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Annals of Surgery

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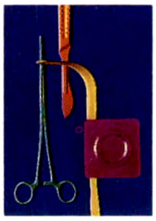

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SCIENTIFIC PAPERS

Intrathymic Islet Transplantation in the Spontaneously Diabetic BB Rat **363**

Pancreatic islet allografts transplanted to the thymus of rats with chemically induced diabetes reverse hyperglycemia and are protected from immunologic rejection. Moreover, recipients of intrathymic islets develop donor-specific unresponsiveness to islets subsequently transplanted to extrathymic sites with no immunosuppression. In spontaneously diabetic BB rats, the thymus also can serve as an effective islet transplant site in protecting islets from destruction by rejection and anti-islet recurrent autoimmunity.

Andrew M. Posselt, B.S., Ali Naji, M.D., PH.D., Jessica H. Roark, B.S., James F. Markmann, M.D., PH.D., and Clyde F. Barker, M.D.
Philadelphia, Pennsylvania

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Hubert J. Stein, M.D., Tom R. DeMeester, M.D., Riccardo Naspetti, M.D., John Jamieson, M.D., and Richard E. Perry, M.D.
Los Angeles, California

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Marc R. Scheltinga, M.D., Lorraine S. Young, R.D., M.S., Kathleen Benfell, R.PH., Rancy L. Bye, R.D., M.S., Thomas R. Ziegler, M.D., Alfred A. Santos, M.D., Joseph H. Antin, M.D., Paul R. Schloerb, M.D., and Douglas W. Wilmore, M.D.
Boston, Massachusetts, and Kansas City, Kansas

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P. R. C. Harvey, PH.D., M.D., R. S. McLeod, M.D., F.R.C.S.(C), Z. Cohen, M.D., F.R.C.S.(C), and S. M. Strasberg, M.D., F.R.C.S.(C)
Toronto, Ontario, Canada

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John H. Pemberton, M.D., *Doris M. Rath* R.N., *and Duane M. Ilstrup,* M.S.
Rochester, Minnesota

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Judah Folkman, M.D., *Sandor Szabo,* M.D., PH.D., *Mark Stovroff,* M.D., *Paul McNeil,* PH.D., *William Li, and Yuen Shing,* PH.D.
Boston, Massachusetts

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Christoph E. Broelsch, M.D., PH.D., *Peter F. Whitington,* M.D., *Jean C. Emond,* M.D., *Thomas G. Heffron,* M.D., *J. Richard Thistlethwaite,* M.D., PH.D., *Larry Stevens,* M.D., *James Piper,* M.D., *Susan H. Whitington, and J. Lance Lichtor,* M.D.
Chicago, Illinois

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Michael W. Mulholland, M.D., PH.D., *Mary S. Sarpa,* R.V.T., *John Delvalle,* M.D., *and Louis M. Messina,* M.D.
Ann Arbor, Michigan

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B. Mark Evers, M.D., *Jin Ishizuka,* M.D., *Courtney M. Townsend, Jr.,* M.D., *Srinivasan Rajaraman,* M.D., *and James C. Thompson,* M.D.
Galveston, Texas

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R. Morton Bolman III, M.D., *Sara J. Shumway,* M.D., *Jorge A. Estrin,* M.D., *and Marshall I. Hertz,* M.D.
Minneapolis, Minnesota

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Robert L. Kormos, M.D., *Harvey S. Borovetz,* PH.D., *John M. Armitage,* M.D., *Robert L. Hardesty,* M.D., *Gary C. Marrone,* M.D., *and Bartley P. Griffith,* M.D.
Pittsburgh, Pennsylvania

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Mark P. Anstadt, M.D., *Michael J. Stonnington,* B.A., *Mark Tedder,* M.D., *Barbara J. Crain,* M.D., PH.D., *Michael F. Brothers,* M.D., *David J. Hilleren,* M.D., *Richard J. Rahija,* D.V.M., PH.D., *J. Alan Menius, Jr.,* M.H.S., *and James E. Lowe,* M.D.
Durham, North Carolina

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Donald L. Morton, M.D., *Leslie Wanek,* DR.PH., *J. Anne Nizze,* P.A.C., *Robert M. Elashoff,* PH.D., *and Jan H. Wong,* M.D.
Los Angeles and Santa Monica, California

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H. Paul Redmond, F.R.C.S.I., *Kenneth D. Chavin,* M.D., *Jonathan S. Bromberg,* M.D., PH.D., *and John M. Daly,* M.D., F.A.C.S.
Philadelphia, Pennsylvania, and Charleston, South Carolina

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C. Gene Cayten, M.D., M.P.H., *William M. Stahl,* M.D., *Nanakram Agarwal,* M.D., M.P.H., *and Jane G. Murphy,* PH.D.
Valhalla, New York

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Mark A. Hardy, M.D., *Nicole Suciu-Foca,* PH.D., *Elaine Reed,* PH.D., *Alan I. Benvenisty,* M.D., *Craig Smith,* M.D., *Eric Rose,* M.D., *and Keith Reemtsma,* M.D.
New York, New York

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Robert W. Bailey, M.D.*, Karl A. Zucker,* M.D.*, John L. Flowers,* M.D.*, William A. Scovill,* M.D.*,*
Scott M. Graham, M.D.*, and Anthony L. Imbembo,* M.D.
Baltimore, Maryland

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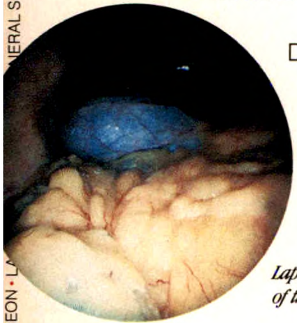

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Andrew L. Warshaw, M.D.
Boston, Massachusetts

WHAT'S NEW IN GENERAL SURGERY

Pancreatic Cancer Cell DNA Content Correlates With Long-term Survival After Pancreatoduodenectomy 648

The DNA content of 47 adenocarcinomas arising in the head of the pancreas was measured. A multivariate life table analysis showed that cellular DNA content is one of the most important predictors of survival after curative pancreatoduodenectomy.

David C. Allison, M.D., PH.D., Kallol K. Bose, PH.D., Ralph H. Hruban, M.D., Steven Piantadosi, M.D., PH.D., William C. Dooley, M.D., John K. Boitnott, M.D., and John L. Cameron, M.D.
Baltimore, Maryland

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Koichi Motojima, M.D., Tsukasa Tsunoda, M.D., Takashi Kanematsu, M.D., Yasuhiko Nagata, M.D., Takeshi Urano, M.D., and Hiroshi Shiku, M.D.
Nagasaki, Japan

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T. Schröder, V. Sainio, L. Kivisaari, P. Puolakkainen, E. Kivilaakso, and M. Lempinen
Helsinki, Finland

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T. V. Taylor, F.R.C.S., *S. Rimmer,* F.R.C.R., *S. Holt,* M.R.C.P., *J. Jeacock,* B.SC., *and S. Lucas,* PH.D.
Manchester, United Kingdom

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Isto H. Nordback, M.D., *Simon MacGowan,* F.R.C.S.I., *James J. Potter,* B.A.,
and John L. Cameron, M.D., F.A.C.S.
Baltimore, Maryland

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Eugene J. Schweitzer, M.D., *Arthur J. Matas,* M.D., *Kristen J. Gillingham,* PH.D., *William D. Payne,* M.D.,
Paul F. Gores, M.D., *David L. Dunn,* M.D., PH.D., *David E. R. Sutherland,* M.D., PH.D.,
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Minneapolis, Minnesota

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Adrian P. Savage, M.A., M.CHIR., F.R.C.S., *and Ronald A. Malt,* M.D.
Boston, Massachusetts

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Abraham Shaked, M.D., PH.D., *and Ronald W. Busuttil,* M.D., PH.D.
Los Angeles, California

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Hans W. Sollinger, M.D., PH.D., *Stuart J. Knechtle,* M.D., *Alan Reed,* M.D., *Anthony M. D'Alessandro,* M.D.,
Munci Kalayoglu, M.D., *Folkert O. Belzer,* M.D., *and John Pirsch,* M.D.
Madison, Wisconsin

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Gurdev Singh, F.R.C.S., Khalil I. Chaudry, Loren C. Chudler, and Irshad H. Chaudry, PH.D.
East Lansing, Michigan

The Effect of Glucocorticoid Administration on Bacterial Translocation: Evidence for an Acquired Mucosal Immunodeficient State 719

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John Alverdy, M.D., F.A.C.S., and Eric Aoys, M.S.
Chicago, Illinois

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Ibrahim M. H. El-Ghazawy, M.D., and S. Eva Singletary, M.D.
Cairo, Egypt, and Houston, Texas

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Martin K. O'Donohoe, M.B., B.SC., F.R.C.S.I., Lewis B. Schwartz, M.D., Zeljko S. Radic, M.D., Eileen M. Mikat, PH.D., Richard L. McCann, M.D., and Per-Otto Hagen, PH.D.
Durham, North Carolina

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David Calcagno, M.D., John W. Hallett, Jr., M.D., David J. Ballard, M.D., PH.D., James M. Naessens, M.P.H., Kenneth J. Cherry, Jr., M.D., Peter Gloviczki, M.D., and Peter C. Pairolero, M.D.
Rochester, Minnesota

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A prospective study was performed on the ability of Doppler arterial pressure measurement to detect the presence of occult arterial injuries in traumatized extremities. Sensitivity and specificity were high, suggesting that this rapid, inexpensive, and noninvasive diagnostic technique may rival arteriography in its ability to identify silent extremity arterial injuries.

Kim Lynch, M.D., and Kaj Johansen, M.D., PH.D.
Seattle, Washington

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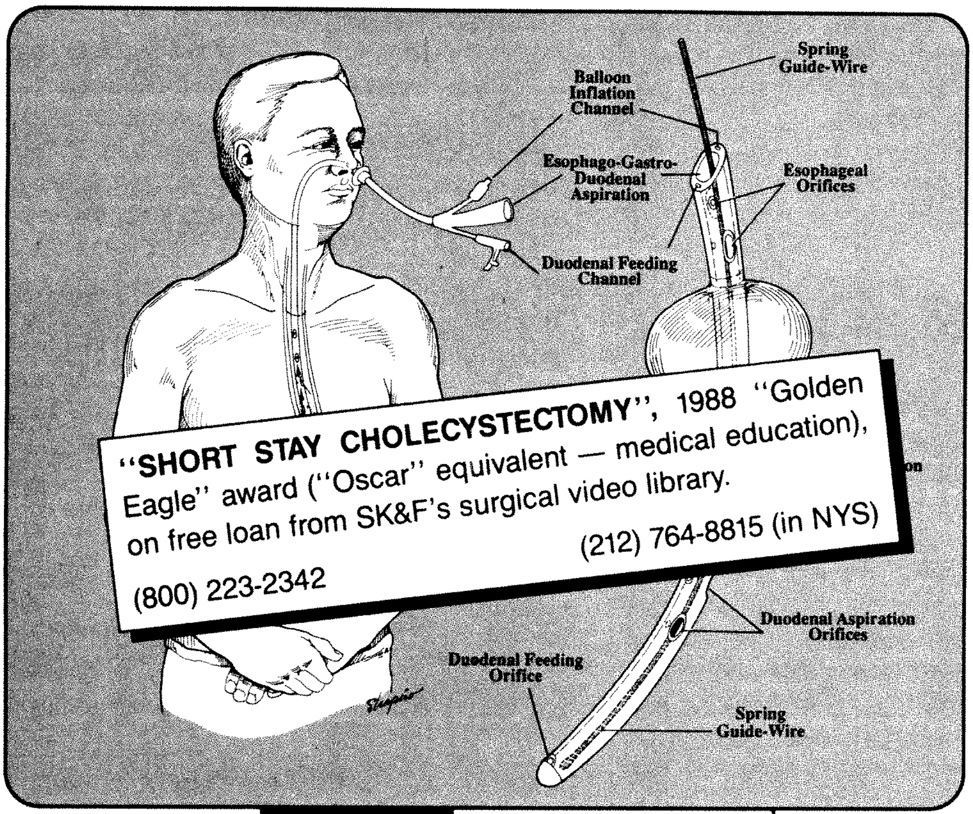

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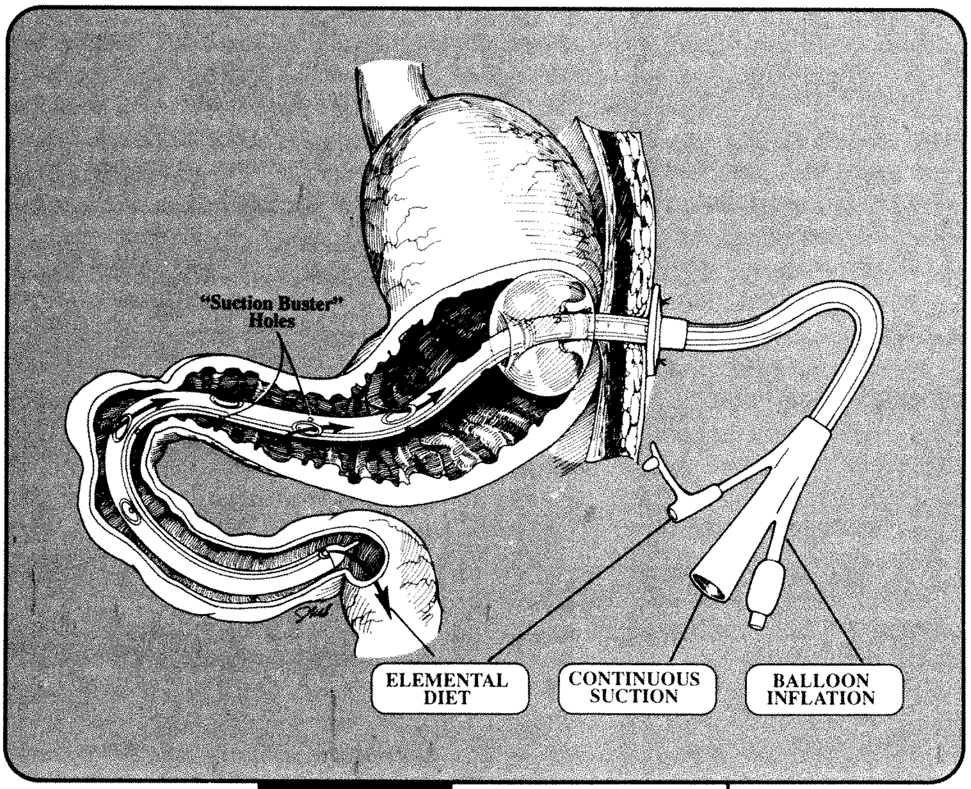

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Intrathymic Islet Transplantation in the Spontaneously Diabetic BB Rat

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Recently it was demonstrated that pancreatic islet allografts transplanted to the thymus of rats made diabetic chemically are not rejected and induce specific unresponsiveness to subsequent extrathymic transplants. The authors report that the thymus can also serve as an effective islet transplantation site in spontaneously diabetic BB rats, in which autoimmunity and rejection can destroy islets. Intrathymic Lewis islet grafts consistently reversed hyperglycemia for more than 120 days in these rats, and in three of four recipients the grafts promoted subsequent survival of intraportal islets. In contrast intraportal islet allografts in naive BB hosts all failed rapidly. The authors also show that the immunologically privileged status of the thymus cannot prevent rejection of islet allografts in Wistar Furth (WF) rats sensitized with donor strain skin and that suppressor cells are not likely to contribute to the unresponsive state because adoptive transfer of spleen cells from WF rats bearing established intrathymic Lewis islets fails to prolong islet allograft survival in secondary hosts.

THE FAILURE OF currently available immunosuppressive protocols to prolong the survival of pancreatic islet allografts has prompted a search for alternate methods that can permit long-term graft function without the need for chronic immunosuppression. One strategy used with some success in animal systems is the transplantation of endocrine tissue to natural or artificially constructed immunologically privileged sites. While many of the best described privileged sites, such as the anterior chamber of the eye and alymphatic skin flap, are incapable of supporting islet endocrine function, others, such as the cerebral cortex and abdominally displaced testicle, have been shown to permit survival of implanted islets and in addition to protect them from alloimmune rejection.[1-3] Although these techniques deserve consideration because they obviate the need for host immunosuppression or pretransplant immunomodulation of the graft, practical considerations would limit their use in clinical islet transplantation.

Recently we reported that the thymus can serve as a novel transplant site that provides a suitable environment for islet endocrine function and promotes survival of allografts without chronic immunosuppression of the recipient. Furthermore animals bearing established intrathymic islet grafts are rendered specifically unresponsive to donor alloantigens as demonstrated by their inability to reject donor-strain islets transplanted to an extrathymic site.[4]

In the present study, we have evaluated further the mechanisms responsible for prolonged survival of intrathymic grafts and for the induction of specific tolerance that follows intrathymic islet transplantation. In addition we have determined the outcome of intrathymic islet transplantation in spontaneously diabetic BB rats, in which both rejection and recurrent anti-beta cell autoimmunity can destroy islet allografts.[5-7]

Materials and Methods

Animals

BB rats were bred and maintained in the animal facilities of the University of Pennsylvania. In our subline of diabetes-prone rats, spontaneous diabetes has a cumulative incidence of 50% to 60% and develops between 60 to 120 days of age. Rats that were considered to have

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developed diabetes (serum glucose levels more than 300 mg/dL on 3 consecutive days) received daily injections of protamine zinc insulin to prevent ketoacidosis and death. Insulin therapy was discontinued after islet transplantation so that blood glucose levels could serve as an index of islet graft survival. Spontaneous recovery from diabetes in BB rats has never occurred in our colony. All BB rats are homozygous for the RT1^u haplotype at the major histocompatibility complex (MHC) locus.

Inbred Wistar Furth (WF, RT1^u) and Lewis (RT1^l) rats were purchased from Harlan-Sprague Dawley (Walkersville, MD) and Charles River Laboratories (Wilmington, MA), respectively.

Induction of Chemical Diabetes

Hyperglycemia was induced in nondiabetic WF rats by a single intravenous injection (65 mg/kg) of streptozotocin. Only rats with nonfasting blood glucose levels of more than 300 mg/dL were considered suitable for transplantation with pancreatic islets.

Islet Isolation and Transplantation

Islets were isolated by collagenase digestion of the pancreas followed by centrifugation through a discontinuous Ficoll gradient as previously described.[8] Contaminating nonislet tissue was removed under a dissecting microscope, and only islets free of adherent acinar, vascular, and lymphoid elements were used for transplantation. Spontaneously diabetic animals received islet grafts 2 to 7 days after onset of diabetes, while chemically induced diabetic rats were allowed to remain hyperglycemic for 6 to 14 days to exclude the possibility that insufficient streptozotocin had been administered to cause permanent diabetes. Freshly isolated islets (1000 to 1500) were transplanted into the following sites: (1) the liver *via* portal vein embolization, (2) the renal subcapsule, (3) the abdominally displaced testicle, and (4) the thymus by injection of 600 to 800 islets into each lobe. Islets were never maintained in tissue culture before transplantation. Hyperglycemia usually was reversed within 1 day and always within 4 days after transplantation. Where noted recipients received 1 mL of rabbit anti-rat lymphocyte serum (ALS) (Accurate Chemical and Scientific Co., Westbury, NY) intraperitoneally at the time of transplantation. Nonfasting blood glucose levels were determined three times each week in all islet recipients, with cessation of graft function being defined as the first of 2 consecutive days of recurrent hyperglycemia (blood glucose level more than 200 mg/dL). At the conclusion of each study, animals were killed and islet-bearing organs were removed, fixed in Bouin's solution, and processed for light microscopy. Serial sections of each organ were stained with hematoxylin and eosin as well as with aldehyde-fuchsin for identification of islet tissue.

Monoclonal Antibodies (MAb) and Flow Cytometry

The following murine mAbs specific for rat lymphocyte markers were used: R73 (anti-α/β T-cell receptor [TCR]; all T cells), OX-8 (anti-CD8; cytolytic/suppressor T cells and NK cells), W3/25 (anti-CD4; helper T cells and macrophages), and Mar 18.5 (anti-surface immunoglobulin, (sIg); B cells). The antibodies were characterized previously.[9,10] Monoclonal reagents were used either as undiluted culture supernatants or obtained in purified form from Bioproducts for Science, Inc. (Indianapolis, IN).

For immunofluorescent analysis, 1 to 2 × 10^6 cervical and mesenteric lymph node cells (LNC) were incubated for 60 minutes with saturating concentrations of the primary mAb, washed twice, and treated for 60 minutes with fluorescein isothiocyanate-conjugated F(ab')$_2$ goat anti-mouse IgG (Tago, Inc., Burlingame, CA). All incubations were performed at 4 C in Dulbecco's phosphate-buffered saline (D-PBS) containing 0.01% sodium azide. Ten thousand viable cells were analyzed for relative fluorescence using a dual laser fluorescence-activated cell sorter (FACS) with logarithmic amplifiers (FACS IV; Becton-Dickinson, Sunnyvale, CA). Background fluorescence was calculated using cells incubated with the fluorescein conjugate alone.

Adoptive Transfer Studies

Spleens from long-term (more than 200 days) WF recipients of intrathymic Lewis islet allografts were teased into single-cell suspensions, washed twice in D-PBS, and injected intravenously (250 to 300 × 10^6 cells/recipient) into sublethally irradiated (450 R), streptozotocin-diabetic WF rats. Twenty-four hours later, animals received fresh Lewis islet allografts beneath the renal capsule without immunosuppression. Control animals received spleen cells from unmanipulated WF rats.

Skin Grafting

Skin grafting was performed according to the method of Billingham.[11] Rejection was defined by extensive graft necrosis and sloughing, as judged by daily inspection after dressings were removed on the seventh day.

Results

Survival of Islet Allografts in Nonimmunosuppressed Spontaneously Diabetic BB Rats

Pancreatic islets isolated from Lewis donors and transplanted by portal vein inoculation into six spontaneously diabetic BB rats were destroyed promptly in every instance (median survival time [MST], 9 days). In this model it is

TABLE 1. *Survival of MHC-compatible and -incompatible Islet Allografts in BB Rats*

Transplant Site	Donor Strain (MHC)	Days of Allograft Survival (MST)*
Liver (intraportal)	Lewis (RT1^l)	8, 9, 9, 19, 24, (9)*
Renal subcapsule	Lewis (RT1^l)	41, 47, 59, >70† >120 × 2 (>64.5)
	WF (RT1^u)	23, 64, >120 (64)
Thymus	Lewis (RT1^l)	>50 × 5, >120 × 6 (>120)‡
	WF (RT1^u)	>120 × 5 (>120)

impossible to distinguish rejection from autoimmune damage, which in itself can cause islet failure. As we have found previously, islets transplanted beneath the renal capsule had a more prolonged and variable survival in BB rats, which are known to be significantly immunodeficient.[12,13] Of the 6 recipients of renal subcapsular grafts, 3 underwent islet failure after 41, 47, and 59 days, 1 died while still normoglycemic after 70 days, and 2 others remained normoglycemic for more than 120 days before they were used for other experiments (see below). In contrast to the results in other transplant sites, none of 11 BB rats that received intrathymic Lewis islets destroyed their grafts. Serum glucose levels in these animals returned to normal within 48 to 72 hours after islet transplantation and remained between 80 and 110 mg/dL for the duration of the study. Six of these animals, after being followed for more than 120 days, were then studied histologically and/or used for other experiments. Five other normoglycemic animals are still being followed for periods between 50 and 67 days. These findings are summarized in Table 1.

If autoimmune diabetes is an MHC-restricted process, islet allografts from MHC-compatible donors, although less susceptible to rejection by BB recipients than those from MHC-incompatible donors, would be more likely to be destroyed by autoimmunity.[14,15] Therefore to investigate whether the thymus protects MHC-compatible islet grafts from rejection and, more importantly, also from autoimmune damage, we compared the outcome of intrathymic and renal subcapsular grafts of WF islets in spontaneously diabetic BB rats. All five recipients of intrathymic WF islets maintained normal serum glucose levels for more than 120 days, while two of three recipients of renal subcapsular WF grafts became hyperglycemic after 23 and 64 days (Table 1).

Assessment of Unresponsiveness in Islet Allograft Recipients

To determine whether intrathymic islets transplanted to diabetic BB rats would induce an unresponsive state that could protect subsequent extrathymic islets from either rejection or autoimmunity, five BB rats that had harbored an intrathymic Lewis islet transplant for 120 days received another Lewis islet allograft transplanted intraportally. No immunosuppression was given at any time. All animals remained normoglycemic and 60 to 65 days after the second allograft the thymus containing the first allograft was removed in four of these rats to allow functional assessment of the extrathymic islet transplant (Fig. 1). Three of the four animals remained normoglycemic after thymectomy while one (BB#1) became hyperglycemic (blood glucose level, 250 to 300 mg/dL) although it remained healthy and continued to gain weight, unlike

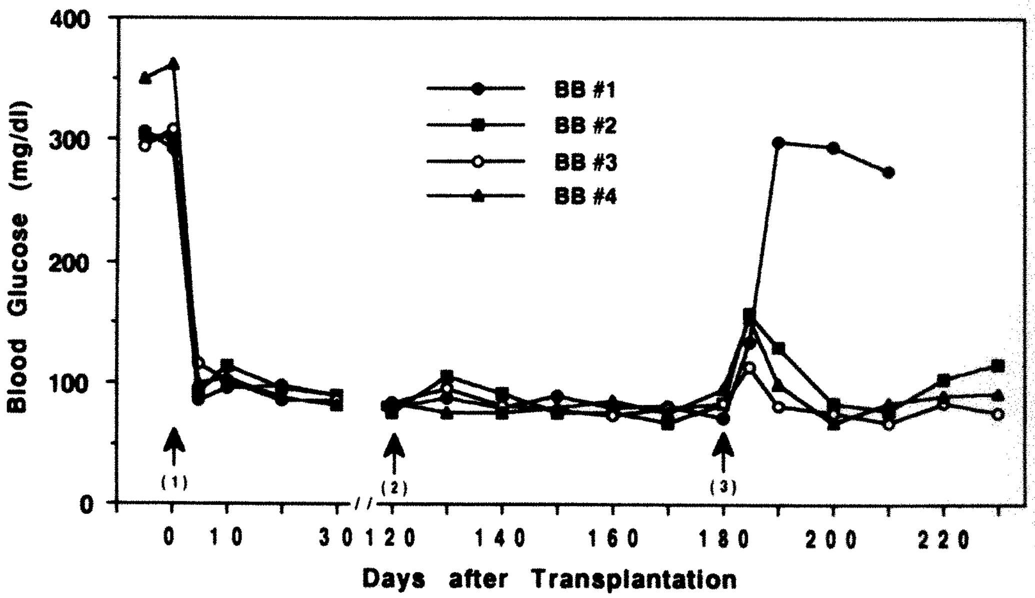

FIG. 1. Blood glucose profiles of spontaneously diabetic BB rats (BB # 1-4) transplanted with Lewis islet allografts. (1) Intrathymic islet transplantation; (2) second transplantation of Lewis islets intraportally; (3) removal of islet-bearing thymus.

untreated BB rats, which usually do not survive without exogenous insulin treatment.

In all six recipients of intrathymic Lewis islets, histologic examination of the thymus after 120 days revealed healthy, well-granulated islets and no lymphocytic infiltrate. In the animals that had remained normoglycemic after thymectomy, the liver, when finally examined histologically, also contained well-granulated noninfiltrated islets (Fig. 2). The liver of the one rat that became hyperglycemic after thymectomy contained intact islets with

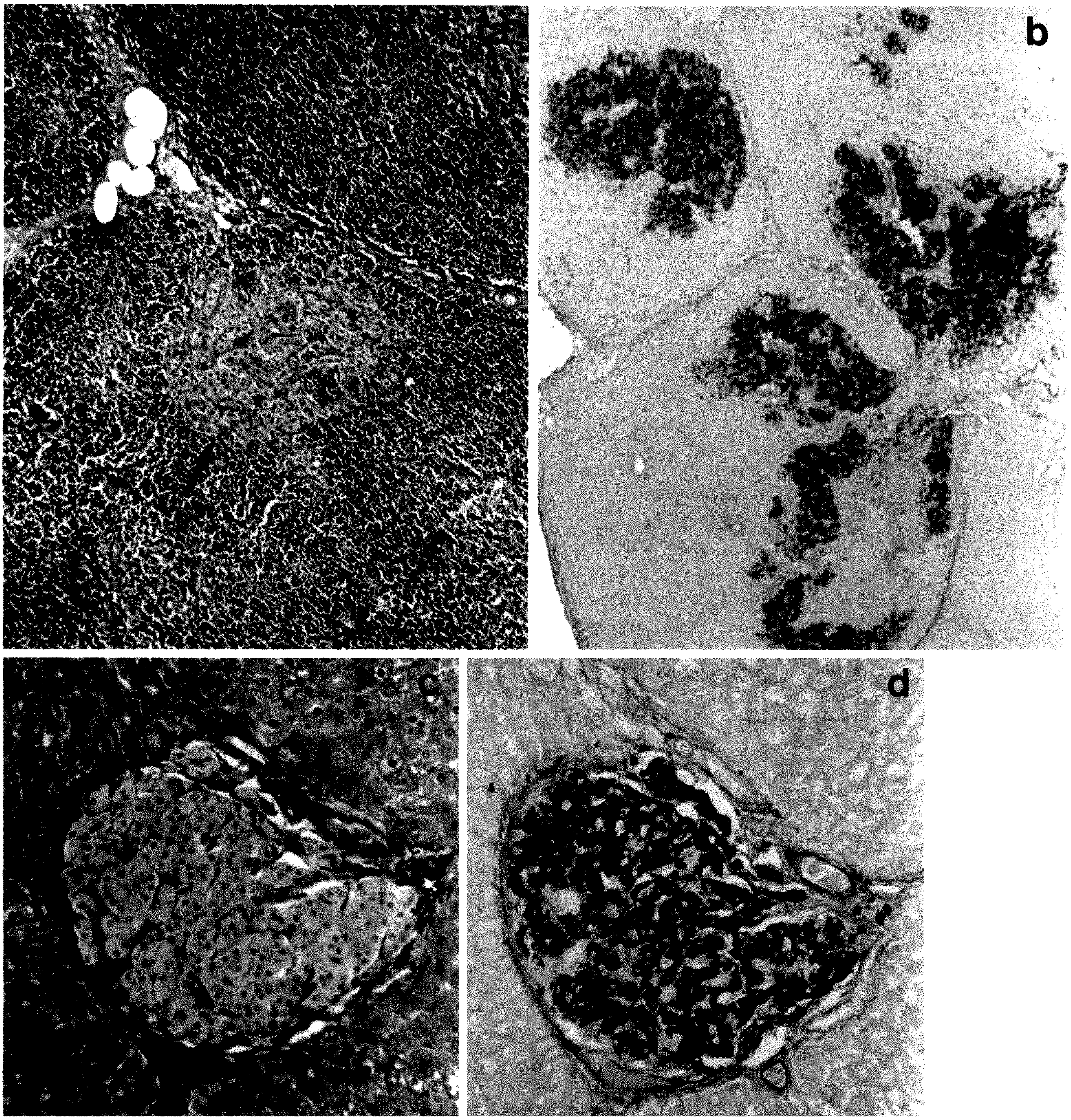

FIG. 2. Photomicrographs of thymus and liver from BB recipients of Lewis islet allografts. (a) Section of a thymus removed 180 days after implantation of Lewis islets. The islets (arrow) appear healthy and there is no mononuclear infiltration (H&E ×80). (b) Aldehyde-fuchsin-stained section from the same specimen demonstrating abundant insulin granules (×20). (c) Section of liver from a rat that was transplanted with intraportal Lewis islets 120 days after intrathymic islet transplantation. Removal of the liver was performed 110 days after intraportal islet transplantation (H&E ×160). (d) The presence of beta cells in the same section is indicated by insulin-specific staining (aldehyde-fuchsin ×160).

no suggestion of insulitis; however they stained weakly for insulin. This histologic finding suggested that an insufficient number of islets had been transplanted to the liver to allow the intraportal graft alone to maintain normoglycemia after removal of the thymus. Such grafts are likely to show degranulation of beta cells.[16]

Although most renal subcapsular transplants in BB rats eventually fail, in this study two rats maintained functional kidney subcapsular islets for more than 120 days, allowing us to assess the capacity of allografts in this site to induce unresponsiveness. One of these rats was grafted with Lewis skin, which was rejected after 29 days, provoking rejection of the islet graft 9 days later. The other rat received a second Lewis islet allograft, transplanted intraportally. This animal remained normoglycemic, and 60 days later was killed for histologic examination of the transplanted islets. Although noninfiltrated islets were found under the renal capsule, only a few recognizable islets remained in the liver, and these were infiltrated by mononuclear cells. Thus it appeared that the persistence of allogeneic islets in the kidney does not diminish the vigor of the immune response to subsequent allografts of donor-strain skin or extrathymic islets. The pancreata of all animals mentioned above were examined histologically and in no instance did recognizable beta cells remain.

Studies of Intrathymic Islet Allografts in Immunized Hosts

To evaluate the capacity of the thymus to act as a privileged site in presensitized hosts, four WF rats were sensitized with Lewis skin allografts. After rejection of the skin grafts, the rats were rendered diabetic with streptozotocin and 5 days later transplanted with Lewis islets into either the thymus or another privileged site (the testicle), in conjunction with 1 mL of ALS administered intraperitoneally. All animals became normoglycemic after transplantation but rejected the islets rapidly (within 10 days), indicating that the efferent arc of the immune response is intact for both intratesticular and intrathymic grafts and confirming our previous experience with allografts in other privileged sites (Table 2).[17]

The vulnerability of established intrathymic islet allografts to immunity induced by subsequent donor strain skin also was assessed. Three (two WF, one BB) rats that had maintained functional intrathymic Lewis islet allografts for 120 to 200 days were grafted with Lewis skin. Skin allograft survival in the two WF rats was minimally if at all prolonged (11, 14 days *versus* 9 to 10 days in six controls). This was also the finding in the BB recipient, in which rejection of the skin was complete in 30 days (control MST: 32 days, n = 4). Interestingly none of these animals rejected the skin grafts in an accelerated manner, indicating that they had not been sensitized by the intrathymic islets and suggesting interruption in the afferent arc of the immune response. The fate of the long-standing intrathymic islets was variable in these same animals after they were challenged with a skin allograft. In the BB rat, diabetes recurred 36 days after skin grafting (6 days after the completion of skin graft rejection). One of the WF rats became hyperglycemic 11 days after skin grafting, while the other has remained normoglycemic despite rejection of the donor-strain skin allograft.

TABLE 2. *Survival of Lewis Islet Allografts in Sensitized WF Recipients*[*]

	Days of Allograft Survival	
Transplant Site	Sensitized Hosts[*]	Unsensitized Hosts
Testicle	10, 10	50 × 2, 76, 110, >200 × 2
Thymus	5, 6	28, 33, 57, >200 × 10

[†] All recipients received 1 cc ALS administered intraperitoneally at time of islet transplantation.

[*] Islets were transplanted 5 days after rejection of a Lewis skin allograft.

In Vitro *Assays to Characterize the Unresponsive State Induced by Intrathymic Islet Allografts*

Several assays were performed to define possible differences in the immune system induced by the presence of alien antigen in the thymus. To exclude the possibility that inoculation of islets into the thymus had disrupted T-cell maturation and thus rendered the animals immunodeficient, lymph node cells from long-term (more than 120 days) BB or WF recipients of intrathymic islets were analyzed by flow cytometry (Fig. 3). Cells from unmanipulated and intrathymically grafted WF rats showed similar percentages of TCR+, CD8+, CD4+, and sIg+ subsets. While both naive and transplanted BB rats demonstrated the T-cell lymphopenia characteristic of this strain,[18] no significant phenotypic differences were noted between these two groups.

Mixed lymphocyte culture assays previously performed on LNC from WF recipients of intrathymic Lewis islets showed normal proliferative responses to donor alloantigens as compared to nontransplanted controls.[4] We did not perform these assays in BB rats because there is never a significant *in vitro* proliferative response to allogeneic stimulators.[18,19]

Adoptive Transfer Studies to Assess Possible Suppressor Cell Activity in Intrathymic Islet Recipients

Two mechanisms that could account for the unresponsiveness of long-term recipients of intrathymic allogeneic islets to subsequent extrathymic transplants are (1) deletion or inactivation within the thymus of specific T-cell populations that could otherwise be expected to destroy extrathymic grafts and (2) generation of suppressor T cells.

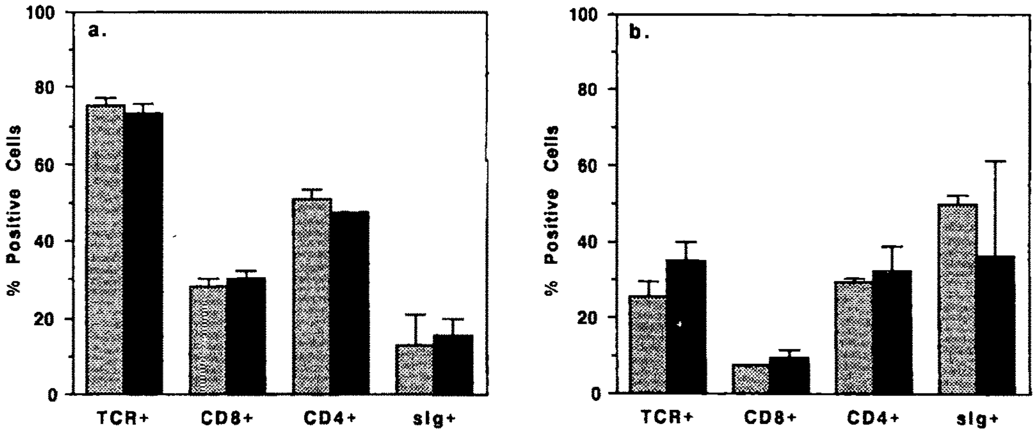

FIG. 3. Immunofluorescent analysis of lymph node cells from WF and BB rats bearing long-term (more than 120 days) intrathymic (IT) Lewis islet allografts. (a) WF controls (shaded bars); WF recipients of intrathymic islets (solid bars). (b) untransplanted spontaneously diabetic BB controls (shaded bars); BB recipients of intrathymic islets (solid bars). Results are means ± SD of three or four separate experiments.

We have already reported the results of studies to assess the first possibility (see Discussion). To evaluate the latter, we adoptively transferred 250 to 300 × 10^6 spleen cells either from nontransplanted WF controls or from WF rats harboring intrathymic Lewis islet allografts for more than 100 days to sublethally irradiated WF hosts. These animals subsequently received islets from Lewis donors beneath the renal capsule. Islet survival in rats given spleen cells from intrathymic recipients was not significantly different than that of the control group and thus provided no evidence for suppressor cells (Table 3).

Discussion

Recently we found the thymus to be another of the relatively few transplant sites that will permit successful engraftment and normal metabolic function of pancreatic islets. Even more interesting was the concomitant observation that the thymus is a previously unrecognized immunologically privileged site. Islet allografts transplanted intrathymically to nonimmunosuppressed rats with chemically induced diabetes exhibited substantially prolonged survival and, if the hosts were briefly immunosuppressed with a single dose of ALS, permanent allograft survival was achieved. Although the precise mechanisms responsible for these findings remain to be elucidated, a partial explanation is suggested by several known morphologic and physiologic characteristics of the organ. Ultrastructural studies as well as experiments using particulate dyes have demonstrated the presence of a blood–thymus barrier surrounding capillaries in the thymic cortex.[20] In addition the thymus possesses no afferent lymphatic supply.[21] These details of vascular anatomy may account for the findings of Michie et al.[22] that there is minimal recirculation of mature T lymphocytes through the thymic parenchyma. Despite the classification of the thymus as a primary lymphoid organ, it is relatively removed from the immune surveillance, that takes place in other tissues, thus explaining the sanctuary it provides for allografts.

In the present study, we sought to determine whether the thymus' immunologically privileged nature would be sufficient to sustain allografts implanted in sensitized hosts that bear an expanded population of effector T cells reactive to donor alloantigens. However, when we transplanted allogeneic islets into the thymus of recipients that had previously rejected donor strain skin grafts, the islets were destroyed in an accelerated manner, demonstrating that the intrathymic site is readily accessible at least to activated T cells. These findings correlate with reports that antigen-stimulated T lymphocytes have an enhanced capacity to recirculate through the thymic parenchyma.[23,24] That the thymus is indeed an immunologically privileged site is not contradicted by these results because it is well established that even sites of proved immunologic privilege such as the anterior chamber of the eye, the brain, and the testicle are incapable of protecting allografts from destruction in previously immunized hosts.[17] Lending further credence to the status of the thymus as a privileged site was our finding that intrathymic islet allografts did not sensitize their hosts to subsequent donor strain test skin grafts, which were rejected with normal tempo. This outcome is consistent with in-

TABLE 3. *Survival of Lewis Islets in WF Rats Inoculated with Syngeneic Spleen Cells from Intrathymic Lewis Islet Recipients*

Status of WF Spleen Cell Donor	Treatment of Recipient*	Days of Islet Allograft Survival
Intrathymic Lewis islets†	450R	8, 9, 12
Naive	450R	12, 14, 16

* Rats were irradiated on day −2 and received 250 to 300 × 10^6 spleen cells on day −1 relative to islet transplantation.

† Animals had retained intrathymic Lewis islet allografts for more than 100 days.

terruption of the afferent arc of the immune response, which is also a characteristic of most other privileged sites.

While the prolonged survival of intrathymic islets in naive hosts may be explained by the immunologically privileged status of the thymus, the finding that recipients bearing established intrathymic grafts are unable to reject subsequent donor strain islets suggests that mechanisms that alter systemic alloimmune responses are also contributory. We have previously demonstrated that frequencies of cytotoxic T-cell precursors specific for donor alloantigens are reduced in intrathymic islet-bearing hosts.[4] Thus one such mechanism may involve deletion or functional inactivation of alloreactive clones by the foreign cells within the thymus. Nevertheless the possibility remains that additional mechanisms such as generation of suppressor or anti-idiotypic T cells may contribute to the acceptance of extrathymic grafts. In fact suppressor cell-mediated unresponsiveness has been shown to develop in several models of long-term graft survival, particularly those in which acceptance of the allograft is achieved by administration of potent immunosuppressive agents.[25,26] Our finding that islet allograft survival in secondary hosts was not prolonged by transfer of lymphoid cells from intrathymic islet-bearing recipients argues against the generation of regulatory cell populations as the basis for the survival of intrathymic islets and further supports the conclusion that the unresponsive state to extrathymic grafts is mediated primarily by deletion or inactivation of specific T-cell clones within the thymus. That skin allograft survival was not prolonged by the intrathymic islets was somewhat disappointing because it indicates that the degree of unresponsiveness we have induced by this method is not comparable to that which is caused by neonatal inoculation of donor-strain bone marrow, and thus should probably not be referred to as "tolerance."[27]

A central focus of the present study was to test the capacity of the thymus to protect islets transplanted to spontaneously diabetic BB rats. It is important to remember that these transplants were done in hosts that had already destroyed their native pancreatic islets. In this sense they were presensitized to islet tissue and presumably had an expanded population of effector cells reactive to putative autoantigens in the transplanted islet tissue.[28] The barrier of autoimmune damage to islets may, in fact, be at least as important to the success of islet transplantation as rejection because in both animals and humans it can result, by itself, in islet failure.[29] Viewing the experiment in this context, it is rather surprising that the outcome of intrathymic islet transplantation was so good. In fact in none of the 11 acutely diabetic BB rats transplanted with Lewis islets was there ever an indication from blood glucose values or histology that even mild rejection or autoimmune insulitis occurred. In contrast all intraportally transplanted islets were rapidly and completely destroyed. In addition to these controls, an extensive collective experience exists from our previous work and that of others concerning the fate of islets transplanted to other extrathymic sites in BB rats (reviewed in Markmann et al.[29]). We and others have noted that transplanted islets are destroyed by BB rats even when the contribution of allogeneic rejection is eliminated by pretransplant *in vitro* culture or by the induction of neonatal tolerance. Thus in no previous reports, despite the fact that BB rats are relatively immunodeficient, could failure from rejection or autoimmune damage be consistently prevented unless islets were subjected to pretransplant culture or unless heavy or continuous immunosuppression was used. For example, when Selawry et al.[30,31] transplanted islets to a privileged site (the testicle) of BB rats, they found it necessary to use either pretransplant tissue culture or ALS (or both) to obtain prolonged survival. Neither pretransplant culture or immunosuppression was used at any stage in the experiments in BB rats reported here.

The possibility that autoimmune destruction of islets is an MHC-restricted process raises another interesting question: Will MHC-compatible islets fare worse than MHC-incompatible ones in BB rats because MHC restriction might protect the latter from autoimmune destruction?[12] Regardless of this consideration, we found the intrathymic site to be protective because both uncultured WF and Lewis islets implanted there uniformly survived while those transplanted to the renal subcapsule or liver did not.

While the prolonged survival of intrathymic islets in BB hosts must, at least in part, be due to the immunologically privileged status of the thymus, the finding that recipients bearing established intrathymic grafts fail to destroy subsequent extrathymic islets either by rejection or autoimmunity argues that additional mechanisms that alter systemic immune responses are also involved. Although the evidence for this presented here in BB rats is good, it is not quite as firm as that in our previous report on the weakening of the alloimmune response we caused in chemically diabetic rats. In three of the four BB rats that were retransplanted with intraportal islets after prolonged residence of an intrathymic graft, the persistence of normoglycemia after removal of the thymus demonstrated that the intraportal graft was not damaged by either rejection or autoimmunity, a conclusion later confirmed histologically. In the fourth rat, however, removal of the thymus did cause hyperglycemia. Examination of the liver of this rat revealed many islets that stained only weakly for insulin granules. No mononuclear infiltrate was noted. This finding is compatible with either of the following explanations: (1) the number of islets transplanted intraportally was insufficient to maintain normoglycemia and the remaining beta cells degranulated; (2) there was specific autoimmune destruction of islet beta cells while the nonbeta cell populations were protected from rejection by the influence of the intrathymic allograft.

Somewhat surprisingly and in contrast to our earlier experiments in streptozotocin-diabetic WF rats, it was not necessary in BB rats to use any immunosuppression to achieve permanent survival of intrathymic islet allografts or to induce unresponsiveness to subsequent extrathymic ones. A possible explanation for this is that the profound T-cell lymphopenia characteristic of BB rats allowed intrathymic islets to survive long enough to effect unresponsiveness. However, despite the immunodeficient status of BB rats, the eventual destruction of the majority of intraportal and renal subcapsular islet allografts demonstrates that these animals retain the capacity to respond to foreign tissue grafts and further underscores the superior nature of the thymus as a transplantation site.

We previously postulated that the donor-specific unresponsive state induced by intrathymic islet transplantation develops as T lymphocytes maturing in the thymus become tolerant to the antigens present within this specialized microenvironment. In animals that are fully immune competent, this effect may require transient depletion of mature peripheral T cells to prevent destruction of the intrathymic graft. However, if prolonged allograft survival in the thymus is achieved on some basis other than immunosuppression, gradual physiologic replacement of the peripheral T-cell repertoire by newly matured T cells may produce similar results. Although evidence exists that a sizeable percentage of peripheral T cells are relatively long lived,[32] the prolonged interval between intrathymic grafting and subsequent intraportal retransplantation (more than 120 days) used in our studies of BB rats may have been sufficient for extensive repopulation of the periphery with "tolerant" lymphocytes. On the other hand, it is possible that, in contrast to our findings using ALS-treated rats, the presence of intrathymic grafts in nonimmunosuppressed hosts can lead to development of regulatory cell populations that inhibit immune responsiveness to donor alloantigens. We are currently addressing this possibility.

This report provides further evidence that the success of intrathymic transplantation of islet allografts depends on the immunologically privileged status of the thymus as well as on systemic alterations in the immune system that this approach induces. Furthermore our demonstration that intrathymic islet transplantation can restore permanent euglycemia in spontaneously diabetic BB rats indicates that the thymus can protect islets from both allogeneic rejection and destruction by recurrent anti-beta-cell autoimmunity. The applicability of this approach to the treatment of human type I (insulin-dependent) diabetes mellitus remains to be determined.

Acknowledgments

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References

1. Barker CF, Naji A, Markmann JM. Transplantation of pancreatic islets. *In* Cerrilli J, ed. Organ Transplantation and Replacement. Philadelphia: JB Lippincott, 1987, pp 575–591.
2. Selawry HP, Whittington K. Extended allograft survival of islets grafted into intra-abdominally placed testis. Diabetes 1984; 33: 405.
3. Tze WJ, Tai J. Allotransplantation of dispersed single pancreatic endocrine cells in diabetic rats. Diabetes 1988; 37:383–392.
4. Posselt AM, Barker CF, Tomaszewski JE, et al. Induction of donor-specific unresponsiveness by intrathymic islet transplantation. Science 1990; 249:1293–1295.
5. Prowse SJ, Bellgrau D, Lafferty KJ. Islet allografts are destroyed by disease occurrence in the spontaneously diabetic BB rat. Diabetes 1986; 35:110–114.
6. Werriger EJ, Like AA. Immune attack on pancreatic islet transplants in the spontaneously diabetic Biobreeding/Worcester (BB/W) rat is not MHC restricted. J Immunol 1985; 134:2383–2386.
7. Naji A, Silvers WK, Bellgrau D, et al. Prevention of diabetes in rats by bone marrow transplantation. Ann Surg 1981; 328–338.
8. Lacy PE, Kostianovsky MH. A method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 1967; 16: 35–39.
9. Mason DW, Arthur RP, Dallman JR et al. Functions of the T-lymphocyte subsets by means of monoclonal antibodies. Immunol Rev 1983; 74:57.
10. Hunig T, Wallmy HJ, Hartley JK, et al. A monoclonal antibody to a constant determinant of the rat T cell antigen receptor that induces T cell activation. J Exp Med 1989; 169:73–86.
11. Billingham RE. Free skin grafting in mammals. *In* Billingham RE, Silvers WK, eds. Transplantation of Tissues and Cells. Philadelphia: Wistar Institute, 1961, p 1.
12. Woehrle M, Markmann JF, Beyer K, et al. The influence of the implantation site (kidney capsule vs. portal vein) on islet survival. *In* Hormone and Metabolic Research. New York: Thieme-Stratton Stuggart, 1990, pp 163–165.
13. Woehrle M, Markmann JF, Armstrong J, Naji A. Effect of transplant site on islet allograft survival in BB rats. Transplant Proc 1987; 19:925–927.
14. Woehrle M, Markmann JM, Silvers WK, et al. Transplantation of cultured pancreatic islets to BB rats. Surgery 1986; 100:334–340.
15. Hegre OD, Enriquez AJ, Ketchum RJ, et al. Islet transplantation in spontaneously diabetic BB/Wor rats. Diabetes 1989; 38:1148–1154.
16. Terasa R, Lacy PE, Hauptfeld V, et al. The effect of cyclosporin-A, low temperature culture and anti-Ia antibodies on prevention of rejection of rat islet allografts. Diabetes 1986; 35:83–88.
17. Barker CF, Billingham RE. Immunologically privileged sites. *In* Kunkel HG, Dixon FJ, eds. Advances in Immunology. New York: Academic Press, 1977.
18. Naji A, Silvers WK, Kimura H, et al. Analytical and functional studies of T cells of untreated and immunologically tolerant diabetes prone BB rats. J Immunol 1983; 130:2168–2172.
19. Georgiou HM, Bellgrau D. Thymus transplantation and disease prevention in the diabetes-prone Bio-breeding rat. J Immunol 1989; 142:3400–3405.
20. Raviola E, Karnovsky MJ. Evidence for a blood-thymus barrier using electron-opaque tracers. J Exp Med 1972; 136:466.
21. Weiss L, ed. The Blood Cells and Hematopoietic Tissues. New York: McGraw-Hill, 1977, pp 503–522.

22. Michie SA, Kirkpatrick EA, Rouse RV. Rare T cells migrate to and persist in normal mouse thymus. J Exp Med 1988; 168:1929.
23. Fink PJ, Bevan MJ, Weissman IL. Thymic cytotoxic lymphocytes are primed *in vivo* to minor histocompatibility antigens. J Exp Med 1984; 159:436.
24. Naparstek Y, Holoshitz J, Eisenstein S, et al. Effector T lymphocyte line cells migrate to the thymus and persist there. Nature 1982; 300:262.
25. Rosen BJ. Cellular mechanisms in neonatal and adult tolerance. Immunol Rev 1989; 107:179–202.
26. Hall BM, Pearce NW, Gunley KE, Dorsch SE. Specific unresponsiveness in rats with prolonged cardiac allograft survival after treatment with cyclosporine. J Exp Med 1990; 171:141–157.
27. Billingham RE, Brent L, Medawar PB. "Actively acquired tolerance" of foreign cells. Nature 1953; 172:603.
28. Rabinovitch A, Pidul C, Baqnenizo H, Machrcy P. Immunological mechanisms of islet B-cell destruction: cytotoxic cells and cytokines. *In* Shafri E, Renold AE, eds. Lessons from Animal Diabetes II. London: John Libbey and Co. Ltd, 1988, pp 52–57.
29. Markmann JF, Brayman KL, Choti MA, et al. Pancreatic transplantation in the spontaneously diabetic rodent. *In* Morris PJ, Tilney NL, eds. Transplantation Reviews, Vol. 21. Philadelphia: WB Saunders, 1988, pp 87–105.
30. Selawry H, Fojaco R, Whittington K. Intratesticular islet allografts in the spontaneously diabetic BB/W rat. Diabetes 1983; 34:1019–1024.
31. Selawry H, Fojaco R, Whittington K. Extended survival of MHC-compatible islet grafts from diabetes—resistant donors in spontaneously diabetic BB/W rat. Diabetes 1987; 36:1061–1067.
32. Freitas AA, Rocha B, Contintro AA. Lymphocyte population kinetics in the mouse. Immunol Rev 1986; 91:5–37.

Discussion

Dr. Anthony P. Monaco (Boston, Massachusetts): It is a pleasure for me to discuss Dr. Naji and Dr. Barker and their group's paper. I have had the privilege of also reading it last night. This is a provocative and outstanding and highly intelligent work. It is a continuum of their extraordinary work over the years, and they never disappoint us. This work is another example of their extraordinary scientific ability and intellectuality.

In their paper today, they have demonstrated that the thymus seems to have the characteristics of a privileged site; that is, that islets placed in the thymus seem not to be rejected. In their manuscript, which I had the opportunity to read, if that experiment is done in a sensitized animal, that is, sensitized to the histocompatibility antigens of the islets placed in the thymus, the experiment does not work, and those islets are rejected.

This is characteristic of the classic privileged site phenomenon. What is different is that residence in the thymus seems to confirm some extrathymic message in the form of extrathymic tolerance. This tolerance, however, also is kind of unique. At least we do not have all the information to determine whether it is typical tolerance of the Billingham-Brent-Medalar type.

First of all when islets were transplanted later extrathymically, they were not rejected as long as islets were in the thymus. We would like to know whether this tolerance extends to similar tissues of the same histocompatibility type, such as skin or liver, or some other tissue, or whether it is unique just for the thymus.

Furthermore this phenomenon still probably needs some extra thymic immunologic ablation because, when it is done not in the BB rat, they have had to use antilymphocyte serum, and, as Drs. Naji and Barker point out in their paper for me, this BB rat has a certain amount of immunodeficiency.

So I have three questions. First have you done any experiments to see whether the tolerance extends to nonislet tissues? Secondly, and most important, if this is an exclusion of the T-cell r ertoire of the MHC of the islet, then it should be able to be accomplished by putting any tissue of the same MHC in that thymus, like lymphocytes, splenocytes, etc.

And then thirdly, does the type of challenge that one does peripherally affect the tolerance that you get? That is if you have more of an immunogenic challenge, is the tolerance broken?

It is a pleasure for me to discuss this extraordinary paper.

Dr. Frank Thomas (Greenville, North Carolina): Our group has also been interested in this area, and the mechanism by which a striking tolerance to islets is achieved, the subject addressed by these studies. We agree that suppressor cells are unlikely to explain this phenomenon.

Cells reactive to donor islets developed by both positive and negative selection in the thymus, an organ described as the place T cells go for their college education. As first described by Miller, Bevan, and others, a number of cells can act within the thymus to execute what has been called a veto phenomenon, effectively vetoing the antidonor activity of a developing thymic cell.

Although commonly involving T cells, the veto mechanism can involve macrophage and dendritic cells, cells acting in concert, some think even with somatic donor cells, which of course would include donor islets. Our group has identified the veto phenomenon in a model of adult tolerance we have studied, and found it to be a quite potent mechanism.

The key feature of the veto mechanism is the ability to signal developing T cells within the thymus to abrogate their programmed cytotoxicity to the donor or veto antigens. Many of the features described by Dr. Naji's group, including clonal deletion or energy as shown by MLC and CTL precursor studies, fit the veto phenomenon to a T.

The uniqueness of the thymus among body organs in generating this reaction is also quite in keeping with the veto phenomenon that occurs largely in the thymus by classical thinking.

Have you entertained the veto hypothesis to explain intrathymic-induced tolerance? There are definitive ways by which this hypothesis could be studied beyond phenotypic analysis of total thymus T-cell maturation and MLC studies performed. I wonder if you have pursued any of these studies to date?

Dr. Mark A. Hardy (New York, New York): This is a most exciting and important study, and I would like to join all the others in complimenting the authors on the thorough and complete study, which I had the privilege to review last night in manuscript form.

This approach of intrathymic injection of antigen to induce immunologic unresponsiveness has really a much broader implication in transplantation than just for islet transplantation, as our own work now has shown that the use of intrathymic UVB-irradiated splenocytes permits indefinite cardiac allograft survival.

Another recent paper in *Lancet*, using isolated glomeruli injected intrathymically in a rat model, allowed subsequent donor-type kidney transplant survival in these rats.

It is therefore conceivable that the use of the approach described by the authors may permit specific clinical manipulation of the host to allow prolonged survival of allografts other than islets and thus avoid or at least reduce the toxic side effects of immunosuppression. To be clinically practical, however, the interval between the intrathymic injection and the subsequent grafting must be very short, of the order of a day or two, to adhere to the way that we preserve organs today.

Therefore I would like to ask the authors whether they have tried to shorten the interval between injection and grafting. The interval that they have used is about 100 days. In our experiments, we have used an interval of a week. This question has to be addressed very carefully.

Have they tried this approach in larger animals, as we are looking for a clinical application?

Theoretically what do they think is the role of the donor antigen-presenting cells *versus* the intrathymic antigen-presenting cells in the development of this type of nonself- and self-recognition?

What are the kinetics of the allograft unresponsiveness in this model? Specifically have they removed the thymus at any point before subsequent grafting to see whether the beneficial effect continues? Have they fulfilled in that sense Koch's postulate?

And finally I would like to ask, do they think that the organ-specific antigens play a role in induction of specific organ unresponsiveness?

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I enjoyed reviewing this paper. I think it is one of the most exciting and important contributions, at least in the last year, to the field of transplantation. And I look forward to much more work on this subject in the future.

I want to thank the society for the privilege of the floor.

DR. JOSEPH E. MURRAY (Boston, Massachusetts): I join the others in acknowledging this superb experimental model. I have always been intrigued by the lag time in many aspects of scientific progress. In this instance there is a 19-year interval from the original description by Raviola and Karnovsky of a blood–thymic barrier in the thymic cortical capillaries to its use in experimental models. As soon as I return home, I will look up that original paper to find out just why they were pursuing that particular phase of thymic anatomy.

This time lag is reminiscent of the lag between Lillie's 1916 observation of the placental intermingling of blood in freemartin cattle and the 1945 report of Ray Owens on the immunologic consequences of this anatomic anomaly. Owens' report led directly to the Billingham, Brent, Medawar paper in *Nature* in 1953 on "Actively Acquired Immunological Tolerance in Mice," a foundation piece in transplantation biology, for which Medawar received a Nobel Prize.

Raviola and Karnovsky apparently traced the lymphatics in the thymus and found absence of the efferent component. Like Dr. Monaco I am intuitively attracted to all possibilities in transplantation that might eliminate the need for immunosuppressive drugs. There are hints that naturally occurring immunosuppressive agents may exist. Dr. John Mannick and James Mobray published on such a serum factor years ago.

Sir Roy Calne noted 20 years ago that the pig liver seemed to possess a natural immune tolerance. Currently his group in Cambridge, England have reported in rats that the serum from successfully allografted liver recipients is protective when tranfused into untreated liver recipients. This observation reminds me of Nate Kaliss's enhancing antibody, which protected tumors from being rejected. Recently a Canadian group has reported that human intestinal allografts seem to survive and function better in the presence of a liver allograft. These observations may be clues for the future.

You state in your manuscript that your animals are not tolerant in the Billingham, Brent, Medawar sense. Billingham, Brent, and Medawar used to wonder whether they were producing tolerant cells or tolerant animals. How would you answer that question today on the basis of your current experience?

Finally I was surprised that the renal capsule was partially a privileged site. I enjoyed this paper very much not only for its clinical but also for its biologic implications. The model applications may well become a classic.

DR. JAMES C. THOMPSON (Galveston, Texas): I thought I might break into this Transplantation Mafia here. My ignorance of the thymus amounts to a national resource, but I would like to ask the authors about duration of thymus activity. We know that the thymus gradually stops working after a while; and at what age does it lose this ability to effect this kind of magic transmutation?

And if that has been studied, is there any way to prolong this immune protective function of the thymus, stick in a new one, or anything like that?

DR. JOHN S. NAJARIAN (Minneapolis, Minnesota): The authors have described an intriguing possibility regarding the formation of some form of immunologic tolerance. They have demonstrated that this is an organ-specific phenomenon, because skin graft from a donor of the same strain is rejected.

I did not get a chance to read the manuscript, but I am intrigued by the immunologic question it raises. That is if you implant islets into the thymus, remove the thymus containing the islets, and then challenge the host with islets from a rat of the same strain, do the second islets survive? In other words do you have cellular tolerance to that specific organ? If so that is the first report I have seen of immunologic tolerance in which persistent antigen has not been present.

And so this is my final question: Have you done this experiment and does it work? When you remove the thymus containing the islets, will a second transplant of islets from the same strain survive?

DR. ALI NAJI: Dr. Murray, the origin for the idea of intrathymic cellular transplantation is the classic paper of Billingham, Brent, and Medawar in *Nature* (172:603, 1953). In this landmark report, which I have reread many times, the authors described the concept and the feasibility of induction of neonatal tolerance in mice inoculated with cells of lymphoid and nonlymphoid tissues such as testes and kidneys. Curiously the total number of animals rendered tolerant was actually small. One wonders if the inefficiency of the tolerance-inducing inoculum was due to the proportion of lymphoid and nonlymphoid cells or to the technical difficulty of targeting the cells to the fetus. It is well known that neonatal inoculation of cells of lymphohematopoietic origin routinely produces a high degree of tolerance. We reasoned that the failure of nonlymphoid cells to induce tolerance may have been related to inability of such cells to "home" to the thymus, whereas cells of lymphohematopoietic origin would be expected to migrate to the thymic microenvironment. Thus we initiated experiments to assess whether cells of nonlymphoid origin if implanted in the microenvironment of the thymus would be capable of inducing tolerance.

We have found that the acquisition of peripheral immune unresponsiveness after intrathymic islet inoculation permits indefinite survival of extrathymic islets, but only slight prolongation of skin allografts. It appears that the thymus behaves as a classic immunologically privileged site and is subject to the usual biologic characteristics of such sites, in that prior sensitization of the host with skin allografts precludes prolonged survival of intrathymic islets.

The question of why occasional islet allografts survive permanently in the renal subcapsule without a prior intrathymic implant is intriguing. The duration of these islet survival was variable, however, and on long-term observation they were all rejected. In contrast virtually all intrathymic islets were protected against rejection and produce a remarkably superior degree of blood glucose homeostasis than intraportal or subrenal capsule islet allografts.

Dr. Najarian, you asked whether the peripheral unresponsiveness could be maintained after the removal of the thymus bearing the alloantigen. We have not carried out extrathymic islet transplants after removal of the thymus, but to some extent our work in the BB rats addresses your question. In our experiments the spontaneously diabetic BB rats were transplanted with intrathymic islets and approximately 100 days later were challenged with an extrathymic (intraportal) allogeneic islets. The BB recipients harboring both intrathymic and intrahepatic islet allografts were observed for an additional 2 months before removal of the thymus bearing the islet allografts. In thymectomized hosts observation for an additional month showed no destruction of the extrathymic islets or recurrence of diabetes. Therefore it appears that the continued presence of the thymus bearing allogeneic islets is not necessary for maintenance of the peripheral unresponsiveness.

Dr. Thompson asked whether the involution of the thymus with age influences the survival of the intrathymic islets. We were initially concerned that with the progressive involution of the thymus we might observe destruction of the islets and recurrence of diabetes. The recipients in our studies were all adults, however, and transplants were carried out at 10 to 12 weeks of age. We have now followed other intrathymic islet recipients for more than 2 years, a period close to the life span of the rat, without any recurrent diabetes.

Dr. Hardy, we are delighted that Dr. Remuzzi and his colleagues from Italy (*Lancet* 337:750, 1991) have confirmed our work by their recent report of prolonged survival of renal allografts after intrathymic glomerular transplantation.

The question of the requirements of the donor *versus* recipient antigen-presenting cells is an important one. We have addressed this issue by intrathymic transplantation of *in vitro* cultured islets to delete intra-islet APCs. This strategy permits us to dissect the contribution of islet endocrine cells (expressing class I major histocompatibility complex [MHC] antigens) or the intra-islet antigen-presenting cells (expressing both class I and class II MHC antigens) toward induction of peripheral unresponsiveness. Our preliminary data indicate that APC-depleted intrathymic allografts survive indefinitely. Interestingly APC depletion obviates the need for administration of a single dose of antilymphocyte serum to reduce the peripheral mature T lymphocytes. We have not assessed the precursor frequencies of the cytologic T lymphocyte (CTL) or helper T cells to assess the tolerogenic potential of class I or class II MHC antigen-bearing cells, however.

Dr. Thomas's remarks were related to the veto phenomenon as a mechanism for the induction of peripheral unresponsiveness. The veto phenomenon as described by Fink and Bevan assumes an interaction between two lymphoid cell populations. I am not aware that nonlymphoid cells, such as islet endocrine cells, can participate in the veto network phenomenon. We have assumed that the peripheral unresponsiveness is due to the persistence of the alloantigen-bearing parenchymal cells (islet endocrine cells) in the thymic microenvironment and their influence on maturing thymocytes. There is evidence that maturing thymocytes are more susceptible to tolerance-inducing signals and moreover that "inappropriate" presentation of antigen by nonlymphoid cells induce a state of anergy in T cells.

Dr. Monaco asked about the abolition of the tolerance by reconstitution of the hosts with syngeneic cells. As you know this constitutes a classic method to abolish neonatal tolerance, and we have begun to assess the impact of the syngeneic lymphoid cell reconstitution on abolition of tolerance after intrathymic islet transplantation. We have carried out further studies on the efficacy of intrathymic transplantation of cells of lymphohematopoietic origin to induce peripheral unresponsiveness. Our preliminary results are encouraging, in that intrathymic inoculation of lymphohematopoietic cells possessing tolerogenic potential appears to induce peripheral unresponsiveness toward other tissue and vascularized organ allografts.

Three-dimensional Imaging of the Lower Esophageal Sphincter in Gastroesophageal Reflux Disease

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The resistance of the lower esophageal sphincter to reflux of gastric juice is determined by the integrated effects of radial pressures exerted over the entire length of the sphincter. This can be quantitated by three-dimensional computerized imaging of sphincter pressures obtained by a pullback of radially oriented pressure transducers and by calculating the volume of this image, in other words, the sphincter pressure vector volume. Validation studies showed that sphincter imaging based on a stepwise pullback of a catheter with four or eight radial side holes is superior to a rapid motorized pullback. Compared with 50 healthy volunteers, the total and abdominal sphincter pressure vector volume was lower in 150 patients with increased esophageal acid exposure ($p < 0.001$) and decreased with increasing esophageal mucosal damage ($p < 0.01$). Calculation of the sphincter pressure vector volume was superior to standard techniques in identifying a mechanically defective sphincter as the cause of increased esophageal acid exposure, particularly in patients without mucosal damage. The Nissen and Belsey fundoplication increased the total and intra-abdominal sphincter pressure vector volume ($p < 0.001$) and normalized the three-dimensional sphincter image. Failure to do so was associated with recurrent or persistent reflux. These data indicate that three-dimensional imaging of the lower esophageal sphincter improves the identification of patients who would benefit from an antireflux procedure. Analysis of the three-dimensional sphincter pressure profile should become the standard for evaluation of the lower esophageal sphincter.

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THE LOWER ESOPHAGEAL sphincter is the primary barrier against reflux of gastric juice into the esophagus. The function of the sphincter depends on the resistance it imposes to the flow of gastric contents from the positive intra-abdominal pressure environment into the negative-pressure environment of the chest. Manometric evaluation of the high-pressure zone at the gastroesophageal junction usually is performed by measuring its peak resting pressure or the pressure at the respiratory inversion point and assuming that this reflects sphincter resistance.[1,2] Various studies have confirmed that this single pressure measurement is lower in patients with increased esophageal exposure to gastric juice than in controls and decreases with increasing severity of mucosal injury.[3,4] Despite this correlation this method has been shown to be inadequate to identify individual patients with mechanically defective sphincters because of the large overlap with normal subjects.[5] There remained a large number of patients with adequate sphincter pressure who had increased esophageal acid exposure on 24-hour esophageal pH monitoring.

Subsequent investigations have shown that the position of the sphincter, in other words, how much of the sphincter is exposed to the positive intra-abdominal pressure environment, and the overall length of the sphincter, also contribute to its resistance in that an overall length or abdominal length below the fifth percentile of normal could nullify a normal sphincter pressure.[6,7] Patients with low normal values of each of these components, however, still could have an incompetent sphincter.[5] Consequently the simple measurement of the sphincter's resting pressure, overall length, and abdominal length was still insufficient to identify subtle mechanical defects of the sphincter that may result in increased esophageal acid exposure.

From a mechanical standpoint, the pressures exerted at each point over the entire length and around the circumference of the sphincter must be taken into account as contributing to the overall resistance of a sphincter. Bombeck and co-workers[8] have applied this concept of resistance to the lower esophageal sphincter using a manometry catheter with radially oriented side holes. They

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showed that the volume circumscribed by radial pressures measured over the entire length of the sphincter allowed integration of sphincter length and pressure into one parameter, termed the sphincter pressure vector volume. The sphincter pressure vector volume was found to be superior to all individual parameters in predicting patients with gastroesophageal reflux disease who would not respond to medical therapy.

The rapid pullback technique used by Bombeck et al. to obtain the sphincter pressure vector volume has several shortcomings. First it is cumbersome because it requires the use of a custom-made motor to perform the pullback at a constant speed and sophisticated computer hardware and software for acquisition and analysis of the data. Second, in contrast to a stepwise pullback, it is unable to measure the amount of sphincter affected by the positive intra-abdominal pressure environment. This is because the patient is requested to hold his breath during the pullback. Consequently the contribution of the intra-abdominal portion of the sphincter to its resistance can not be evaluated. Finally the rapid pullback technique requires a special catheter assembly with radially oriented transducers located at the same level to obtain a three-dimensional sphincter image. This does not allow the evaluation of esophageal body function in the same setting, and the patient must go through the inconvenience of being reintubated with a second catheter assembly to complete the esophageal evaluation.

In a validation study, we compared sphincter pressure vector volumes obtained by a rapid and stepwise pullback of a catheter with eight radially oriented side holes located at the same level, and a stepwise pullback of a catheter with four sequential side holes oriented radially at 5-cm intervals. In the latter situation, esophageal body function could be evaluated concomitantly. Based on the validation study, we selected the technique that provided the best discrimination of patients with increased esophageal acid exposure from control subjects. We then compared the sphincter pressure vector volume with standard sphincter analysis in a large number of patients with increased esophageal acid exposure and various degrees of mucosal damage. The effect of the Nissen and Belsey fundoplication on the three-dimensional sphincter pressure profile also was evaluated.

Materials and Methods

Populations and Study Design

The validation population consisted of 15 healthy normal volunteers and 27 symptomatic patients, 17 with normal esophageal acid exposure and 10 with increased esophageal acid exposure documented by 24-hour ambulatory esophageal pH monitoring. All 10 patients with increased esophageal acid exposure had at least 6 months of aggressive acid suppression therapy before the studies and were classified by the referring physician as having gastroesophageal reflux disease unresponsive to medical treatment. On endoscopy 3 of these 10 patients had no mucosal injury, 5 had esophagitis, and 2 had Barrett's esophagus. All volunteers and patients in the validation study underwent three manometric studies to measure the sphincter pressure vector volume, in other words, a rapid and stepwise pullback using a catheter dedicated to measure radial sphincter pressures consisting of eight radially oriented side holes located at one level, and a stepwise pullback using a standard manometry catheter with four sequential side holes oriented radially at 5-cm intervals. The latter catheter is routinely used in manometric studies and allows evaluation of the esophageal body in the same setting. The data obtained were used to assess whether the sphincter pressure vector volume can be reliably obtained with the standard manometry catheter and to identify the best manometric technique to differentiate patients with increased esophageal acid exposure from control subjects.

The method for three-dimensional sphincter imaging selected in the validation study was subsequently compared with standard manometry of the lower esophageal sphincter in a study population consisting of 50 normal healthy volunteers and 150 consecutive patients with increased esophageal acid exposure on 24-hour esophageal pH monitoring. On endoscopy 57 of 150 patients had no mucosal injury, 42 had esophagitis, 20 had a stricture, and 31 had Barrett's esophagus. Thirty-two of these patients also had follow-up studies 1 to 10 years after a Nissen (n = 28) or Belsey (n = 4) fundoplication. The demographic data of the study populations are given in Table 1.

Approval by the local Human Research Ethic Committee was obtained to study the subjects in the validation

TABLE 1. *Demographic Data of the Study Population*

Study	Number	Mean Age (Range)	Male/Female Ratio
Validation study			
Normal volunteers	15	32.2 (22–48)	6/9
Patients, no GERD	17	55.3 (32–69)	10/7
Patients, GERD	10	52.7 (28–68)	6/4
Main study			
Normal volunteers	50	35.2 (23–71)	20/30
Patients with GERD			
No mucosal injury	57	46.2 (16–69)	27/30
Esophagitis	42	48.2 (17–72)	22/20
Stricture	20	59.0 (17–78)	13/7
Barrett's	31	53.1 (25–76)	19/12
Patients s/p antireflux surgery			
Nissen fundoplication	28	51.3 (25–72)	16/12
Belsey fundoplication	4	58.2 (44–72)	2/2

GERD, gastroesophageal reflux disease.

study and the 50 normal volunteers. Written informed consent was obtained from all subjects before the study.

Manometry of the Lower Esophageal Sphincter

Manometry of the lower esophageal sphincter was performed after an overnight fast. Medications known to interfere with gastrointestinal secretory or motor function were discontinued at least 48 hours before the study. Various catheter assemblies with an outer diameter of 4.8 mm and four to eight lateral openings of 0.8 mm diameter were used. The catheters were perfused with distilled water at a constant rate of 0.6 ml/min using an Arndorfer pneumohydraulic low-compliance perfusion pump. Each tube was connected to an external pressure transducer positioned at the midaxillary level. Before each test the transducers were calibrated using a mercury-filled manometer so that a change in pressure of 1 mm Hg corresponded to 0.5 mm on the recording paper. The pressures measured were printed by a Gould ES1000 16-channel recorder on paper running at a velocity of 5 mm/second. A belt pneumograph was positioned around the chest to record respiratory excursions. A piezoelectric transducer was taped to the neck at the level of the cricoid cartilage to indicate pharyngeal swallows. The catheter assembly was passed through an anesthetized nostril into the stomach, and the gastric pressure pattern was confirmed. All pressure measurements were read with the gastric baseline pressure as reference point.

Three-dimensional Imaging of the Lower Esophageal Sphincter

Three-dimensional manometric imaging of the lower esophageal sphincter was performed with a rapid and stepwise pullback of various catheters illustrated in Figure 1.

Rapid motorized pullback manometry was performed using a catheter assembly with eight radially oriented side holes located at the same level in 45-degree angles to each other. The side holes were withdrawn through the gastroesophageal junction at a constant speed of 3.3 mm/second by a custom-made high-torque hysteresis synchronous motor. The subjects were instructed to hold their breath in the end-expiratory position during the pullback. The mechanical pullbacks were repeated until three satisfactory readings were obtained. Sphincter pressures in all eight radial channels were measured manually at 0.5-cm intervals beginning with the rise of pressure above gastric baseline until the recorded pressures in all channels fell below gastric baseline. Because the breath was held during the motorized pullback, the respiratory inversion point and consequently the abdominal portion of the sphincter could not be identified by this technique.

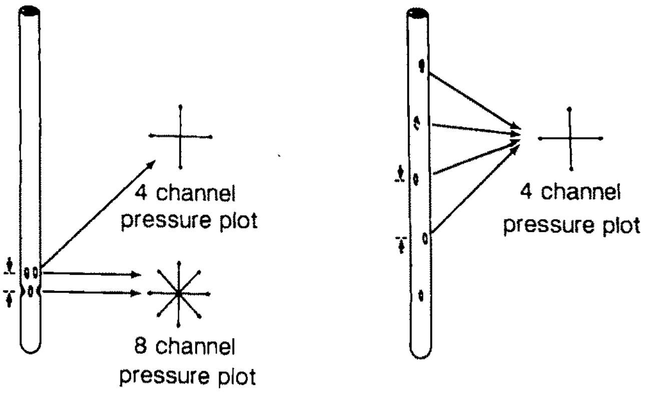


FIG. 1. Catheters used to obtain a three-dimensional sphincter image. (A) A catheter with eight radial side holes at one level oriented in 45° angles to each other. Three-dimensional spincter images were obtained using a rapid and stepwise pullback technique. The sphincter pressure vector volume was calculated based on eight or four radial side holes placed over the length of 1 cm (between arrows). (B) A standard esophageal motility catheter with five side holes spaced at 5-cm intervals (between arrows). The four proximal side holes are oriented in 90° angles to each other. A stepwise pullback of the four proximal side holes through the lower esophageal sphincter was used to construct a three-dimensional sphincter image and calculate the sphincter pressure vector volume.

Stepwise pullback manometry was performed using the catheter assembly with eight radially oriented side holes located at one level in 45-degree angles to each other and the second assembly containing four sequential side holes spaced in 5-cm intervals and oriented radially in 90-degree angles to each other. The latter catheter was identical to the catheter routinely used for standard manometric evaluation of the lower esophageal sphincter and esophageal body. The catheter assembly was withdrawn from the stomach in 1-cm increments every 20 seconds until all transducers had passed through the gastroesophageal junction. The patient was instructed to breathe normally but to avoid swallowing. The stepwise pullback of the catheter with eight radial channels was repeated three times. End-expiratory sphincter pressures in all radial channels were measured manually at 1-cm intervals from the rise of the end-expiratory pressure above gastric baseline until the recorded end-expiratory pressures in all channels fell below gastric baseline. The respiratory inversion point, in other words, the level when the positive deflections in the pressure curve caused by inspiration within the abdomen changed to negative within the chest, was located in each channel and used to identify the intra-abdominal portion of the sphincter.

Three-dimensional images of the lower esophageal sphincter were constructed by a computer program developed by one of us (REP). Pressures measured at each station of the pullback were plotted radially around an axis representing gastric baseline pressure with the program, accounting for the longitudinal spacing of the side holes on the catheter (Fig. 2). For visual purposes the three-dimensional reconstruction of the sphincter pressure

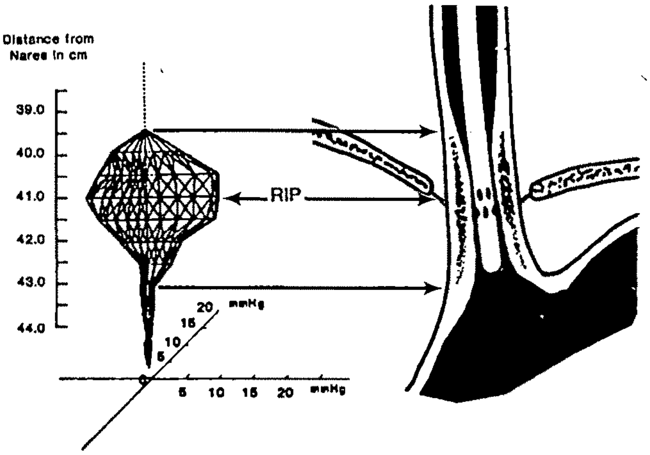


FIG. 2. Computerized three-dimensional imaging of lower esophageal sphincter. A catheter with four to eight radial side holes is withdrawn through the gastroesophageal junction. For each level of the pullback, the radially measured pressures are plotted around an axis representing gastric baseline pressure. When a stepwise pullback technique is used, the respiratory inversion point (RIP) can be identified.

image was enhanced by applying a cubic curve-smoothing interpolation, which retains the original data points while adding intermediate ones to give a smoother surface to the three-dimensional sphincter image and improve its readability. This interpolation was performed for imaging only, whereas all mathematical calculations were made solely with the primary data. The three-dimensional image can be rotated on the computer screen and inspected from various angles (Fig. 3).

The volume circumscribed by the three-dimensional sphincter pressure profile was measured by calculating the cross-sectional radial pressure areas at each measured level, in other words, 5-mm intervals for the rapid pullback and 10-mm intervals for the stepwise pullback, using the trigonometric formula for an irregular octagon or tetragon. Each cross-sectional sphincter pressure area, expressed as square millimeters of mercury, then was multiplied by the length of the section. The sum of all cross-sectional sphincter pressure areas integrates radial pressures exerted over the entire length of the sphincter into one number, the total sphincter pressure vector volume expressed in units of $\text{mmHg}^2 \times \text{mm}$. With the stepwise pullback technique, which allowed identification of the respiratory inversion point, the intrathoracic and intra-abdominal portions of the sphincter pressure vector volume, in other words, the portions of the sphincter pressure vector volume located above and below the respiratory inversion point, also were determined. The pressure vector volume was calculated from data obtained by (1) a rapid and stepwise pullback of a catheter with eight radial side holes located at the same level; (2) a rapid and stepwise pullback of four radial side holes located at the same level; and (3) a stepwise pullback of a catheter with four sequential side holes oriented radially and spaced in 5-cm intervals. Measurement 2 was obtained from recordings made with the eight-channel catheter by ignoring every other channel (Fig. 1). A sphincter was defined as mechanically defective when the total or abdominal sphincter pressure vector volume was below the fifth percentile of that measured in 50 normal volunteers.

Standard Manometry of the Lower Esophageal Sphincter

Standard manometric evaluation of the lower esophageal sphincter was performed using a catheter with five lateral side holes placed at 5-cm intervals from the distal end of the catheter and oriented around the circumference. The catheter was withdrawn from the stomach at 1-cm increments every 20 seconds until all side holes had passed through the gastroesophageal junction. The patient was instructed to breathe normally but to avoid swallowing. Overall length, abdominal length, in other words, length below the respiratory inversion point, and resting pressure of the lower esophageal sphincter were measured as previously described.[5] Based on extensive studies in normal volunteers and patients with increased esophageal acid exposure, the lower esophageal sphincter was defined as mechanically defective if, on the mean of five pullbacks, the resting pressure was below 6 mm Hg, the overall length was less than 2.0 cm, or the abdominal length was less than 1.0 cm.[5,9]

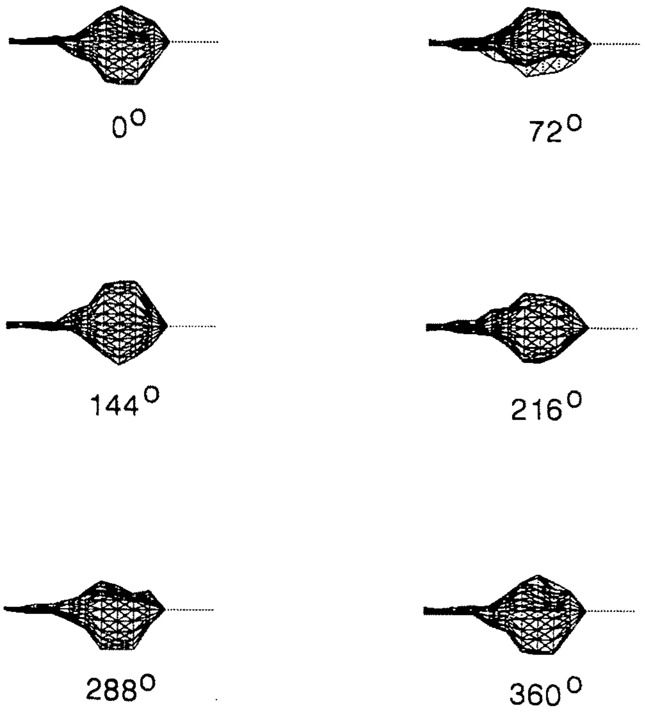


FIG. 3. The three-dimensional sphincter pressure image of a normal volunteer shown from various angles. This effect is achieved by rotating the image around an axis representing gastric baseline. Note the marked asymmetry of the sphincter.

Ambulatory 24-hour Esophageal pH Monitoring

Outpatient 24-hour esophageal pH monitoring was performed using a combined Ingold glass electrode with a built-in reference electrode. The probe was calibrated in standard buffer solutions at pH 7 and 1 before and after the study. Only recordings with an electrode drift of less than 0.2 pH units over the 24-hour monitoring period were accepted. The electrode was passed transnasally and placed 5 cm above the upper border of the lower esophageal sphincter. The electrode was connected to a portable digital data recorder, which stored pH readings every 4 seconds (Synectics, Irving, TX). After placement of the probe, the subjects were sent home and instructed to remain in the upright or sitting position until they retired for the night, to perform normal daily activity but to avoid strenuous exertion, and to follow a diet restricted to three meals composed of food with a pH between 5 and 6. Only water was permitted between meals. A diary was kept of food and fluid intake, symptoms experienced during the monitored period, the time when the supine position was assumed in preparation for sleep, and the time of rising in the morning. All medications known to interfere with foregut motor or secretory function were stopped at least 48 hours before the study. The amount of esophageal exposure to gastric juice ($pH < 4$) was quantitated using a composite scoring system.[9,10] A patient was considered to have increased esophageal exposure to gastric juice if the composite score exceeded the 95th percentile of 50 normal healthy volunteers, in other words, a score value above 14.8.

Endoscopy

Upper gastrointestinal endoscopy was performed in all patients by the senior author (TRD), who was unaware of the results of manometry and pH monitoring at the time of endoscopy. The presence of esophagitis was recognized by mucosal erythema (grade 1), linear erosions and friability (grade 2), or coalescent erosions, the so-called cobblestone mucosa (grade 3). An esophageal stricture was identified by the inability to pass a 12-mm endoscope with ease. Biopsies were performed on all strictures to exclude malignancy, and they were dilated to 50 French before manometry and pH monitoring. There was an interval of at least 1 week between dilatation and esophageal function tests. Barrett's esophagus was diagnosed by histologic documentation of columnar epithelium lining the esophagus at least 3 cm above the endoscopic gastroesophageal junction.

Statistical Methods

The sphincter pressure vector volumes obtained with various techniques in the validation population were compared with each other using Spearman's rank correlation coefficient. The ability of the various techniques to discriminate patients with increased esophageal acid exposure from control subjects was evaluated using analysis of variance and comparison of the confidence intervals.

Standard nonparametric tests for unpaired and paired data sets were used to compare the sphincter pressure vector volumes between or within subject groups of the study population. The prevalence of a mechanically defective sphincter was compared between groups using the Fisher's exact test of proportion. A $p\text{-value} < 0.05$ was considered significant. Unless otherwise stated, all data are expressed as mean ± standard error of the mean (SEM).

Results

The validation study showed that measurements of the sphincter pressure vector volume with eight radially oriented side holes at one level compare well with those made with four radial side holes at one level (Table 2). Measurements obtained with the stepwise pullback of a catheter with four or eight radial side holes located at one level were similar to those obtained with a standard manometry catheter having four sequential radial side holes placed in 5-cm intervals (Table 2). This gave confidence in the use of the standard manometry catheter to measure the sphincter pressure vector volume.

Figure 4 shows the individual measurements of the sphincter pressure vector volume made with a rapid and stepwise pullback of the catheter with eight radial side holes at one level and a stepwise pullback with the standard motility catheter. Both stepwise pullback techniques provided a better discrimination of subjects with increased esophageal acid exposure than a rapid pullback. This was reflected also by a higher significance level and a smaller overlap in the confidence intervals between those subjects with and without increased esophageal acid exposure (Table 3).

TABLE 2. *Comparison of Various Techniques to Calculate the Sphincter Pressure Vector Volume*

Technique	Spearman's Rank Correlation Coefficient	p
RP (8 channel) *vs.* RP (4 channel)	0.86	<0.0001
SP (8 channel) *vs.* SP (4 channel)	0.91	<0.0001
SP (8 channel) *vs.* SSP (4 channel)	0.81	<0.0001
SP (4 channel) *vs.* SSP (4 channel)	0.82	<0.0001

RP, Rapid motorized pullback of radial transducers located at one level.

SP, stepwise pullback of radial transducers located at one level.

SSP, stepwise pullback of radial transducers located at 5-cm intervals.

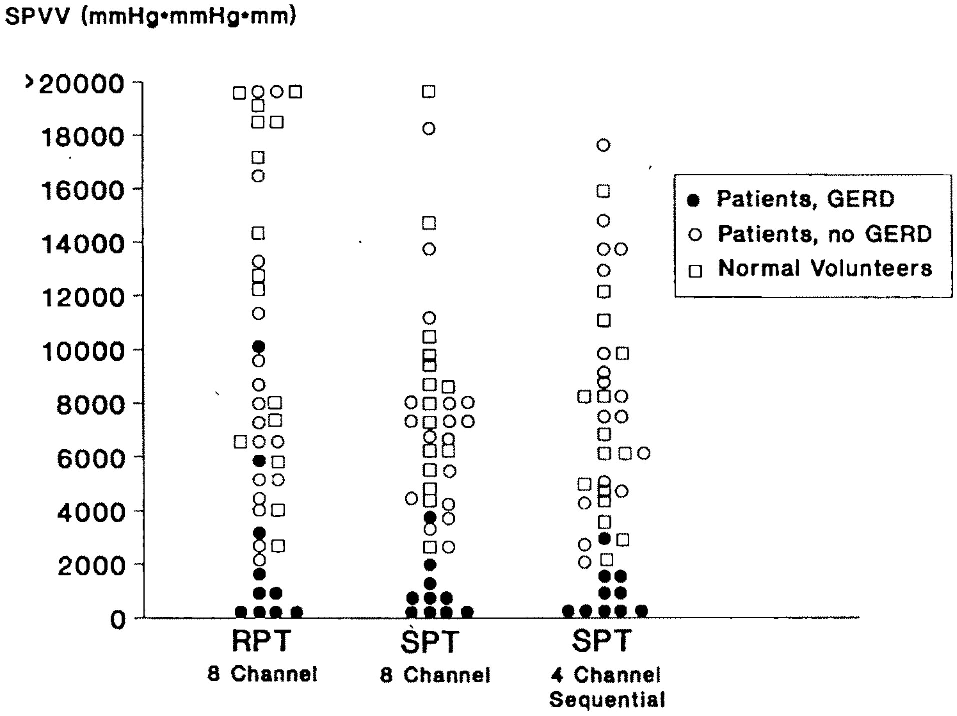


FIG. 4. A plot of the individual sphincter pressure vector volumes (SPVV) obtained with a rapid or stepwise pullback (RPT or SPT) of a catheter with eight radial side holes at one level and a stepwise pullback of a catheter with four sequential radial side holes placed at 5-cm intervals. Both stepwise pullback techniques provided a better discrimination of patients with gastroesophageal reflux disease (GERD) from control subjects than the rapid pullback technique.

Normal values for the total sphincter pressure vector volume, and the intra-abdominal and intrathoracic components obtained in 50 normal volunteers with a stepwise pullback of the standard manometry catheter are given in Table 4. Figure 5 shows that total and intra-abdominal sphincter vector volumes obtained with this technique are significantly lower in patients with increased esophageal exposure to gastric juice as compared with normal volunteers ($p < 0.001$), and decrease with increasing severity of mucosal injury ($p < 0.01$).

TABLE 3. *Mean Sphincter Pressure Vector Volumes and Confidence Intervals in Subjects With and Without Increased Esophageal Acid Exposure*

Technique to Measure SPVV	Normal Esophageal Acid Exposure (N = 27) Mean (99.99% CI)	Increased Esophageal Acid Exposure (N = 10) Mean (99.99% CI)	p
Rapid pullback (8 radial side holes at one level)	10841 (6866–14816)	2340 (0–9451)	0.001
Stepwise pullback (8 radial side holes at one level)	7925 (5485–10366)	872 (0–5237)	0.00001
Stepwise pullback (4 radial side holes located at 5-cm intervals)	7403 (4950–10724)	673 (0–4802)	0.00001

SPVV, sphincter pressure vector volumes; CI, confidence interval.

Figure 6 compares the prevalence of a mechanically defective sphincter based on analysis of the three-dimensional sphincter image, in other words, total and intra-abdominal sphincter pressure vector volume, with standard techniques of sphincter analysis, in other words, sphincter pressure at the respiratory inversion point, overall length, and abdominal length, in patients with increased esophageal exposure to gastric juice and various degrees of mucosal injury. Both techniques show an increasing prevalence of a mechanically defective sphincter with increasing severity of mucosal injury. In patients with increased esophageal exposure to gastric juice but no mucosal injury, analysis of the three-dimensional sphincter image identified a significantly higher prevalence of a defective sphincter as compared with standard techniques ($p < 0.05$). The differences between the two techniques in patients with complications of gastroesophageal reflux disease, in other words, esophagitis, stricture, or Barrett's esophagus, were not significant.

The effect of an antireflux procedure on the three-dimensional sphincter pressure profile in a patient with Barrett's esophagus is shown in Figure 7. Nissen fundoplication restored the sphincter image to normal. Figure 8 displays the mean sphincter pressure vector volume in 32 patients with gastroesophageal reflux disease before and after a Nissen or Belsey fundoplication. Both procedures markedly increased the total and intra-abdominal sphincter pressure vector volume ($p < 0.001$). The total

TABLE 4. *Normal Values for Sphincter Pressure Vector Volume (SPVV) Obtained with a Stepwise Pullback of Four Radially Oriented Transducers Placed at 5-cm Intervals*

SPVV	Mean	Standard Error	Median	5th Percentile	95th Percentile
Intraabdominal	3613	531	2012	684	12918
Intrathoracic	2050	319	1452	476	6022
Total	5723	843	3667	1212	16780

sphincter pressure vector volumes before and after antireflux surgery for the individual patients are shown in Figure 9. Preoperatively 26 of 32 patients had a defective total sphincter pressure vector volume, whereas three of six of the remaining patients had an isolated defect in the intra-abdominal pressure vector volume. Subjective and objective control of reflux was associated with a normalization of the sphincter pressure vector volume in 28 patients. Four patients had persistent or recurrent reflux on 24-hour esophageal pH monitoring after the antireflux procedure. In three fourths of these patients, the postoperative sphincter pressure vector volume was below the 5th percentile of normal volunteers.

Discussion

Gastroesophageal reflux disease is a common foregut disorder that is complicated by esophagitis, stricture, or Barrett's esophagus in about 50% of affected patients.[9] A mechanically defective lower esophageal sphincter is the cause of increased esophageal acid exposure in the majority of patients with complications of the disease.[11] Medical therapy in this situation is plagued by high failure and relapse rates.[12,13] This is because medical therapy is aimed at suppression of acid secretion while allowing other noxious ingredients of gastric juice like pepsin, trypsin, and bile salts to reflux unabated through the defective sphincter, resulting in persistent mucosal injury. Even if medical therapy is effective in healing mucosal damage, the mechanical defect of the sphincter persists and mucosal injury recurs as soon as medical therapy is discontinued.[12-14] Conversely antireflux surgery is designed to correct a mechanically defective sphincter and can effectively prevent reflux of any gastric content.[15] It is important to identify the patients with a mechanically defective lower esophageal sphincter before the development of complications to avoid the loss of esophageal body function, which is known to occur as mucosal injury progresses.[16] In such patients surgery should be performed before the loss of esophageal body function occurs.[17,18] The present study shows that measuring the sphincter pressure vector volume is superior to standard techniques in identifying a mechanically defective lower esophageal sphincter as the cause of increased esophageal acid exposure, particularly in patients with no mucosal injury.

Radial manometric evaluation of lower esophageal sphincter pressures is not new. Using a stepwise pullback of a catheter with radial side holes, Winans[19] demonstrated a marked cross-sectional asymmetry of the lower esophageal sphincter in normal volunteers. Three-dimensional images of the lower esophageal sphincter in healthy volunteers obtained by a rapid pullback of a catheter with eight radial side holes were recently presented by Bemel-

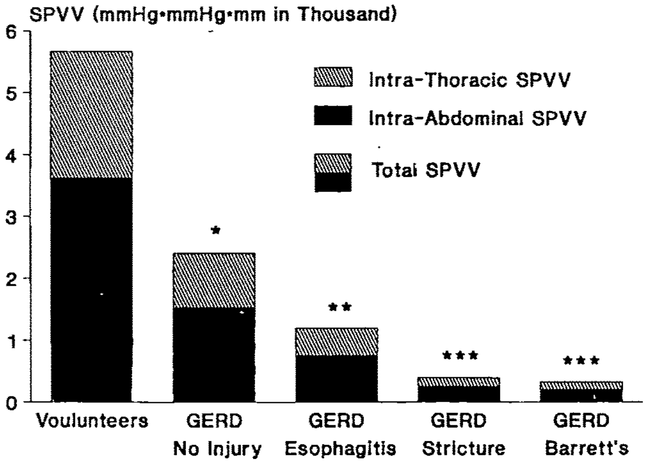


FIG. 5. Mean total and intra-abdominal SPVV in 50 healthy volunteers and 150 patients with gastroesophageal reflux disease GERD and various degrees of mucosal damage. The p values are given for total and intra-abdominal SPVV. $^*p < 0.01$ *versus* volunteers; $^{**}p < 0.01$ *versus* volunteers and GERD patients with no mucosal injury; $^{***}p < 0.01$ *versus* all other groups.

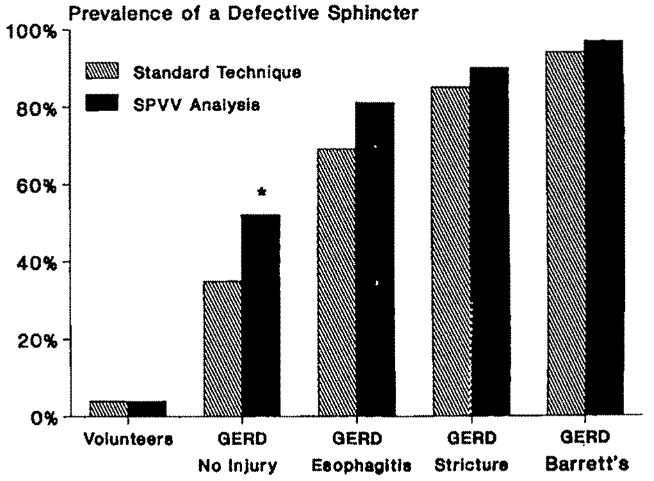
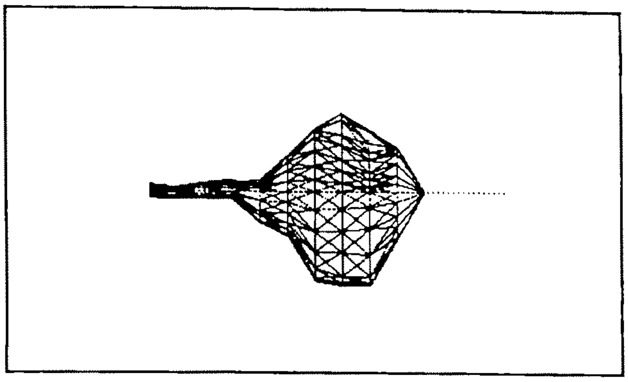
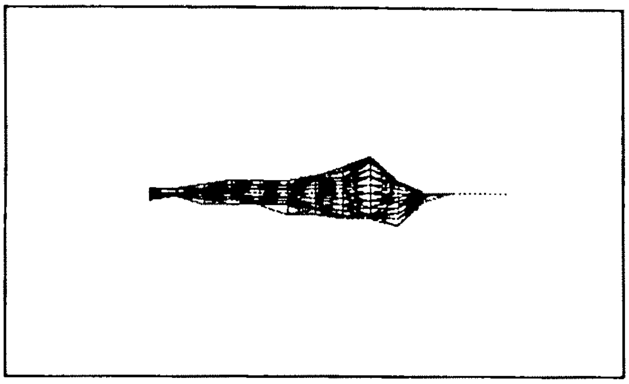


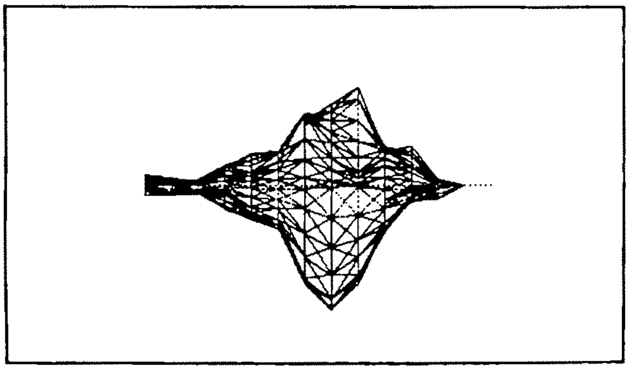
FIG. 6. Comparison of standard manometric techniques and SPVV analysis in the identification of a mechanically defective lower esophageal sphincter. $^*p < 0.05$ *versus* standard manometry.



Normal



Barrett's Preop



After Nissen

FIG. 7. The three-dimensional sphincter pressure image in a normal volunteer, a patient with Barrett's esophagus, and the same patient after Nissen fundoplication.

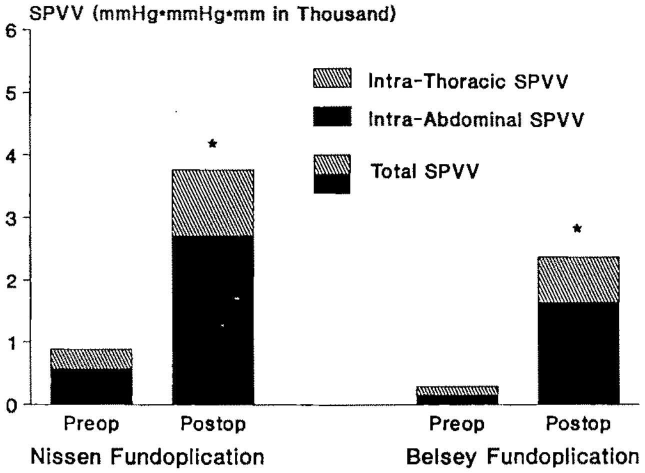


FIG. 8. Effect of the Nissen (N = 28) and Belsey (N = 4) fundoplication on the SPVV. *p < 0.001 *versus* preoperative values. SPVV, sphincter pressure vector volume.

man et al.[20] The use of radially measured sphincter pressures in the assessment of patients with gastroesophageal reflux disease was first reported by Bombeck et al.[8] Applying sophisticated computer technology, he and his coworkers analyzed the three-dimensional sphincter pressure profile obtained by a rapid pullback of a catheter with four to six radial side holes located at one level. They introduced the "sphincter pressure vector volume" as a parameter that integrates sphincter pressures exerted around the circumference and along the entire length of the sphincter into one value representing lower esophageal sphincter resistance. In all these studies, radial sphincter pressures were measured with a dedicated catheter assembly that did not allow the concomitant assessment of esophageal body function. The present study showed that four radially oriented side holes are sufficient to reliably evaluate the sphincter pressure vector volume and that a stepwise pullback technique with a catheter containing radial side holes located at the same level or placed se-

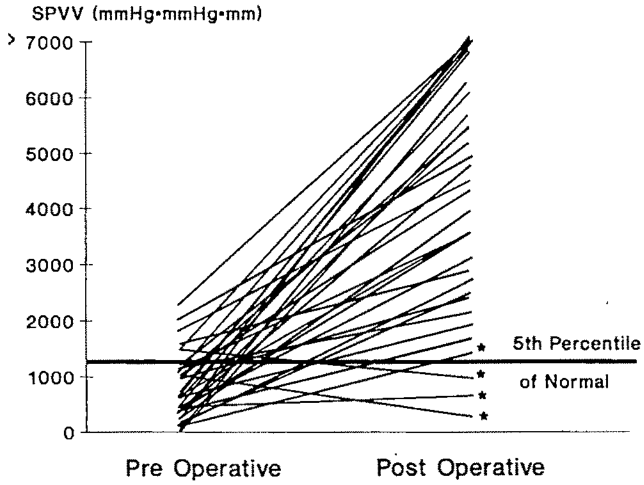


FIG. 9. Individual preoperative and postoperative sphincter pressure vector volumes (SPVV) in 32 patients undergoing antireflux surgery. *Patients with persistent or recurrent reflux. The six preoperative values above the 5th percentile line are six patients who had isolated abnormality in their abdominal SPVV but a normal total SPVV.

quentially in 5-cm intervals is superior to a rapid pullback in discriminating patients with gastroesophageal reflux disease from control subjects. Consequently a stepwise pullback of a catheter assembly with four sequential radial side holes was used in all subsequent studies. This catheter assembly allows evaluation of esophageal body function in the same setting. In addition calculation of the sphincter pressure vector volume by this technique is less complex and does not require the sophisticated computerized data acquisition and analysis systems used by other groups.

Evaluation of the three-dimensional sphincter pressure profile in a large population of patients with increased esophageal acid exposure showed that both total and abdominal sphincter pressure vector volume were markedly lower compared with 50 healthy volunteers, and decreased with increasing severity of mucosal injury. Although it is tempting to ascribe the loss of sphincter function to inflammation or tissue damage, the observation of a low sphincter pressure vector volume in the absence of mucosal damage suggests that the loss of sphincter resistance is primary and probably due to smooth muscle abnormalities.[21]

Standard techniques to assess sphincter resistance and calculation of the sphincter pressure vector volume showed an increasing prevalence of a mechanically defective sphincter with increasing severity of mucosal injury. Calculation of the sphincter pressure vector volume did not have a significant advantage over standard manometric techniques in detecting a defective sphincter in patients with advanced complications of gastroesophageal reflux disease, but significantly increased the sensitivity of manometry in identifying a mechanically defective sphincter in patients with increased esophageal acid exposure but no mucosal damage. This indicates that standard techniques, in other words, measurement of sphincter pressure, overall length, and abdominal length, can reliably identify gross sphincter defects but are insufficient to detect subtle sphincter abnormalities.

Despite the advance of three-dimensional sphincter imaging in assessing sphincter resistance, there still remains a number of patients with increased esophageal acid exposure and an apparently normal lower esophageal sphincter. A marked asymmetry of the sphincter, which is not taken into account when calculating the sphincter pressure vector volume, may be responsible for reflux in some of these patients.[22] In the remainder other causes of increased esophageal exposure to gastric juice are likely to be present. In such patients a careful evaluation of esophageal body function, gastric emptying, gastric acid secretion, and duodenogastric reflux should be performed.[9]

In patients with increased esophageal acid exposure due to a mechanically defective lower esophageal sphincter, reconstruction of a functional sphincter by an antireflux procedure provides the only rational therapy in that it effectively abolishes reflux of any gastric contents in over 90% of patients.[15] Our study demonstrates that this is achieved by increasing the total and abdominal sphincter pressure vector volume to normal. This is further emphasized by the observation that failure to restore the three-dimensional sphincter pressure profile to normal was associated with persistent or recurrent reflux.

In summary the present study shows that the analysis of the three-dimensional sphincter pressure profile obtained with a stepwise pullback is superior to a rapid pullback technique in the ability to differentiate between subjects with or without increased esophageal acid exposure. Calculation of the sphincter pressure vector volume increases the sensitivity of manometry to identify a mechanically defective lower esophageal sphincter as the cause of increased esophageal acid exposure, particularly in patients with no mucosal injury. The effect of an antireflux procedure in controlling reflux is dependent on restoration of the sphincter pressure vector volume to normal. Calculation of the sphincter pressure vector volume allows identification of patients who would benefit from an antireflux procedure before the development of mucosal injury and loss of esophageal body function. Consequently this manometric technique should become the standard for the evaluation of the lower esophageal sphincter in patients with gastroesophageal reflux disease.

References

1. Winans CS, Harris LD. Quantitation of lower esophageal sphincter competence. Gastroenterology 1967; 52:773–778.
2. Castell DO, Richter JE, Dalton CB, eds. Esophageal Motility Testing. New York: Elsevier, 1987.
3. DeMeester TR, Wernly JA, Bryant GH, et al. Clinical and in vitro determinants of gastroesophageal competence: A study of the principles of antireflux surgery. Am J Surg 1979; 137:39–46.
4. Haddad JE. Relation of gastroesophageal reflux to yield sphincter pressures. Gastroenterology 1970; 58:175–184.
5. Zaninotto G, DeMeester TR, Schwizer W, et al. The lower esophageal sphincter in health and disease. Am J Surg 1988; 155:104–111.
6. O'Sullivan GC, DeMeester TR, Joelsson BE, et al. The interaction of the lower esophageal sphincter pressure and length of sphincter in the abdomen as determinants of gastroesophageal competence. Am J Surg 1982; 143:40–47.
7. Bonavina L, Evander A, DeMeester TR, et al. Length of the distal esophageal sphincter and competency of the cardia. Am J Surg 1986; 151:25–34.
8. Bombeck CT, Vaz O, DeSalvo J, et al. Computerized axial manometry of the esophagus. Ann Surg 1987; 206:465–472.
9. DeMeester TR, Stein HJ. Gastroesophageal reflux disease. *In* Moody FG, Carey LC, Jones RC, et al, eds. Surgical Treatment of Digestive Disease, 2nd ed. Chicago: Year Book Medical Publishers, 1989, pp 65–108.
10. DeMeester TR, Wang CI, Wernly JA, et al. Technique, indications and clinical use of 24-hour esophageal pH monitoring. J Thorac Cardiovasc Surg 1980; 79:656–667.
11. Stein HJ, Barlow AP, DeMeester TR, et al. Complications of gastroesophageal reflux disease: Role of the lower esophageal

sphincter, esophageal acid and acid/alkaline exposure, and duodenogastric reflux. Arch Surg 1991 (in press).

12. Hetzel DJ, Dent J, Reed WD, et al. Healing and relapse of severe peptic esophagitis after treatment with Omeprazole. Gastroenterology 1988; 95:903–912.
13. Lieberman DA. Medical therapy for chronic reflux esophagitis: Long term follow up. Arch Intern Med 1987; 147:1717–1720.
14. Howard TM, Frei JV, Flowers M, et al. Omeprazole heals esophagitis but does not improve abnormal motility in reflux esophagitis. Gastroenterology 1990; 98:61(A).
15. DeMeester TR, Bonavina L, Albertucci M. Nissen fundoplication for gastroesophageal reflux disease: Evaluation of primary repair in 100 consecutive patients. Ann Surg 1986; 204:9–20.
16. Stein HJ, Eypasch EP, DeMeester TR, et al. Circadian esophageal motor function in patients with gastroesophageal reflux disease. Surgery 1990; 108:769–778.
17. Salama FD, Camant G. Long term results of the Belsey Mark IV antireflux operation in relation to the severity of esophagitis. J Thorac Cardiovasc Surg 1990; 100:517–519.
18. Stein HJ, DeMeester TR. Who benefits from antireflux surgery? World J Surg 1991 (in press).
19. Winans CH. Manometric asymmetry of the lower esophageal sphincter. Dig Dis Sci 1977; 22:348–354.
20. Bemelman WA, Van der Hulst VPM, Diykhuis T, et al. The lower esophageal sphincter shown by computerized representation. Scand J Gastroenterol 1990; 25:601–608.
21. Biancani P, Zabinsky MP, Behar J. Pressure, tension, and force of closure of the human lower esophagal sphincter and esophagus. J Clin Invest 1975; 56:476.
22. Crookes PF, Kaul BK, DeMeester TR, et al. Manometry of individual segments of the distal esophageal sphincter and its relation to functional incompetence. Arch Surg 1991 (in press).

DISCUSSION

DR. EDWARD W. HUMPHREY (Minneapolis, Minnesota): Dr. Stein has presented us with a method of mathematically modeling the lower esophageal sphincter area. I have long admired the efforts of Dr. DeMeester's group to raise the work on the lower esophageal sphincter from the realm of metaphysics to that of real science, and this paper is no exception.

I do have three questions on this work. The first is, what is the reproducibility of the sphincter pressure vector volume with time in the same individual? If it is good, it might permit the longitudinal studies to finally learn whether the motor abnormalities seen with esophagitis are the cause or effect of the abnormal reflux.

Secondly I made some extrapolations from the graphs in your manuscript. I found that although the absolute value of the abdominal portion of the pressure vector volume is less in the patients with esophagitis and the total volume is less, the proportion is the same. In patients with esophagitis, 67% of the total volume is below the diaphragm and thus in the abdomen; in your volunteers it was 63%. With the error in my extrapolation, those are essentially the same, and I wonder if you can explain that? Because 80% to 85% of patients with abnormal reflux have a hiatus hernia, I would have expected the abdominal portion to be considerably smaller in patients with esophagitis.

Third have you noted any differences that will predict which of the patients will be in the 10% to 15% that have a poor result from a fundoplication? If you could do that with this method, it would be a significant advance.

Thank you very much.

PROFESSOR MARTIN ALLGOWER (Basel, Switzerland): I have four questions (they are rather naive) and one comment. The Basel anatomist was telling us surgeons that we have a very astounding capacity to name and to cut structures he had never seen. I think it is the merit of Doris Lieberman to describe the anatomic reality of the lower esophageal sphincter.

My first question is, whether sphincter pressure vector volumes in a way do mirror the anatomic findings that Lieberman has been describing? Naturally the actual clinical application of the Lieberman procedure would be somewhat devastating to the patient! I wonder whether you agree that your quantification of LE-function constitute an interesting confirmation of Doris Lieberman's findings.

Second the merits of your three-dimensional imaging are to a large extent validated by their conformity with the increased esophageal acid exposure. Now my question is, what is the additional information with regard to therapeutic decisions taken from your values?

Thirdly the reflux disease without mucosal lesions seems to be the main real case for your method. Now could it be that your method picks out the known hypersecreters who would probably benefit from an early antirefluxplasty?

And fourthly one "philosophical" question: Does not the "amount of subjective suffering" constitute an important element of an indication for operation?

I enjoyed so very much to see Bombeck's finding substantiated and made more easily applicable.

Naturally I was particularly happy to see that the Nissen procedure really has stood the test of time. Thank you very much for this very good paper.

DR. PHILIP DONAHUE (Chicago, Illinois): My only slide—may I see it now, please?—illustrates a concept of the vector volume and introduces my three questions. This is actually one of Tom Bombeck's slides from his presentation here in 1987. A "full-bodied" sphincter is normal; "skinny, abnormal-looking" sphincters, are abnormal. After fundoplication, the contour of the sphincter is more normal.

My first question relates to vector volume: The computer program converts multiple virtual areas of segments of the sphincter into a volume, by multiplying an average area times the length. The radius of that cylinder is the critical factor, and I am concerned about the station pullback technique that you have used. You thought that it was better than rapid pullback technique, but we believe the rapid pullback eliminates subjective factors in estimating the average pressure along sphincters. When station pullback is employed, bias is introduced.

How do you avoid subjective bias in estimating pressures?

Secondly we have found that asymmetry of the sphincter is an important concept (Probably affecting only patients with marginal pressures). Can you tell us whether you have noted the presence of asymmetry in any of your patients with failed sphincter?

Finally the vector volume concept still does not identify 20% of patients who require surgery, because 20% of the patients you operated on had a normal study; we believe that abnormal (but as yet unmeasurable) aspects of gastric components of the reflux barrier can help explain reflux disease in some of these patients. I invite your comments about this possibility.

DR. HUBERT J. STEIN (Closing discussion): Thank you very much for those kind remarks. I would like to answer Dr. Humphrey's questions first. He addressed the reproducibility of the measurement over time. We performed reproducibility measurements within the same subject within 2 or 3 hours. Within this short period, all of the measurements, in other words, the rapid and stepwise pullback, were highly reproducible. We do not have long-term reproducibility studies yet, but it would certainly be interesting to see what they would show.

Why was the percentage of the intra-abdominal segment of the sphincter similar in volunteers and in the patients with esophagitis, even though many of the latter had a hiatal hernia? We have previously shown that the presence of a hiatal hernia does not necessarily mean that there is no intra-abdominal segment of the lower esophageal sphincter. The length of the intra-abdominal segment is determined by the insertion of the phreno-esophageal membrane, and even in patients with a large hiatal hernia, intra-abdominal pressure can be exerted on the sphincter through the hiatus. Patients with esophagitis frequently had an isolated defect in their intra-abdominal segment, however, but there were others in the same group of patients with a normal intra-abdominal segment but a short overall length of the sphincter and a defective total sphincter pressure vector volume.

Based on the three-dimensional sphincter image, can we predict in whom the antireflux procedure is going to fail? We have not gone that far yet, and I do not know whether this method will be able to give us these predictors. The four patients in whom the antireflux procedure failed to restore esophageal acid exposure to normal all had a breakdown of the repair. We assume that all of these patients had a sphincter pressure vector volume immediately after the operation. But the repair broke down over the course of time mostly because of technical factors, and the three-dimensional image was destroyed.

Dr. Allgower mentioned the anatomic studies by Dr. Lieberman, of which I am well aware because I am now working in the same department as Dr. Lieberman. She is pleased to see that our three-dimensional manometric images of the lower esophageal sphincter correspond very well with the anatomic equivalent of the lower esophageal sphincter that she has identified on the dissecting table.

What is the benefit of doing three-dimensional manometry if we can identify the patient with increased esophageal acid exposure with esophageal pH monitoring alone, and what are our indications for antireflux surgery? Manometry of the lower esophageal sphincter is necessary to identify those patients with increased esophageal acid exposure who will benefit from antireflux surgery.

Increased esophageal acid exposure can be caused by a defective lower esophageal sphincter, gastric causes or poor esophageal clearance. In our opinion an antireflux procedure should be performed only in these patients in whom increased esophageal acid exposure is due to a defective lower esophageal sphincter. Those are the only patients who really do benefit from antireflux surgery. It is particularly important to identify those patients with a mechanical defective sphincter who have not yet developed mucosal injury. This is because we know that, with progression of mucosal injury to esophagitis, stricture, and Barrett's esophagus, the motor function of the esophageal body deteriorates and the results of an antireflux procedure becomes less predictable. Three-dimensional manometry of the lower esophageal sphincter is superior to standard manometry, particularly in these patients.

The group of Dr. Donahue and the late Dr. Bombeck, who first presented three-dimensional sphincter images, are proponents of the rapid pullback technique to obtain the image. Because our validation study has shown superiority of the stepwise pullback in discriminating patients with and without reflux disease, we decided to use the stepwise pullback routinely. The stepwise pullback may be superior to the rapid pullback because it allows us to identify the respiratory invasion point and the intra-abdominal segment of the sphincter, which is a very important factor of the antireflux mechanism.

Could asymmetry of the sphincter contribute to its incompetence? I think yes, it does. At the recent Western Surgical Association meeting, we presented a paper on asymmetry of the lower esophageal sphincter. By evaluation of sphincter asymmetry, we can identify another 10% or 12% of patients who have a normal sphincter pressure vector volume but a markedly asymmetric sphincter. We believe that these patients have reflux because of the asymmetry of their lower esophageal sphincter.

Glutamine-enriched Intravenous Feedings Attenuate Extracellular Fluid Expansion After a Standard Stress

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A double-blind, randomized controlled trial was performed to determine the effect of glutamine (GLN)-enriched intravenous feedings on the volume and distribution of body fluids in catabolic patients. Subjects with hematologic malignancies in remission underwent a standard treatment of high-dose chemotherapy and total body irradiation before bone marrow transplantation. After completion of this regimen, they were randomized to receive either standard parenteral nutrition (STD, n = 10) or an isocaloric, isonitrogenous nutrient solution enriched with crystalline L-glutamine (0.57 g/kg/day, GLN, n = 10). Extracellular water (ECW) and total body water (TBW), determined by bromide and heavy water dilution techniques, were measured before the conditioning treatment and after termination of the intravenous feedings that were administered for 27 ± 1 days. In addition electrical resistance (R, in ohms, Ω) and reactance (X_c, Ω) of the body to a weak alternating current were measured at these time points. Both study groups were comparable for age, weight, height, sex, and diagnosis. Initial TBW was highly related to electrical resistance ($r = -0.93$, $p < 0.001$). After conditioning therapy, bone marrow infusion, and intravenous feedings, a 20% expansion in ECW was observed in the STD group (ECW: 18.0 ± 1.1 L *vs.* 14.9 ± 1.0, $p = 0.012$), and this fluid retention was associated with a marked decrease in electrical resistance (R: 514 ± 28 Ω *vs.* 558 ± 26, $p < 0.05$). In contrast the extracellular fluid compartment in patients receiving GLN-supplementation did not change (ECW: 15.8 ± 0.9 L *vs.* 15.4 ± 0.8, $p = 0.49$), and the body's resistance was maintained (R: 552 ± 27 Ω *vs.* 565 ± 23, $p = 0.42$). Expansion of ECW could not be related to differences in fluid or sodium intake, or to the use of diuretics or steroids. Patients receiving the STD solution, however, exhibited a greater number of positive microbial cultures ($p < 0.01$) and a higher rate of clinical infection compared with the GLN patients (5/10 *vs.* 0/10, $p < 0.05$); the fluid expansion in infected STD patients was greater compared with uninfected individuals (Δ ECW: + 5.0 ± 1.4 *vs.* 0.7 ± 0.5, $p = 0.007$). In this model of catabolic stress, fluid retention and expansion of the extracellular fluid compartment commonly observed after standard total parenteral nutrition can be attenuated by administering glutamine-supplemented intravenous feedings, possibly by protecting the host from microbial invasion and associated infection.

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RETENTION OF SODIUM and water and the concomitant expansion of body fluid compartments are characteristic sequelae of injury.[1] These changes are thought to result from the enhanced release of catabolic mediators[2] and hormones such as vasopressin[3] and aldosterone.[4] This hormonal response is proportional to the severity of injury and is accentuated by blood loss[5] and severe infection.[6] Although the cause of the redistribution in body fluids is incompletely understood,[7] it has generally been found that "stress" is associated with expansion of the extracellular fluid compartment.[8]

A regimen of enteral feedings often is not possible in catabolic patients. For example patients undergoing allogeneic bone marrow transplantation (BMT) and associated chemoradiotherapy often exhibit intense nausea, vomiting, oral mucositis, and diarrhea, which preclude adequate enteral food intake.[9] Thus intravenous nutrition is commonly administered in this patient population. Administration of total parenteral nutrition (TPN), however, is associated with expansion of the extracellular fluid compartment that persists at hospital discharge.[10]

Usual intravenous feedings do not contain glutamine (GLN), although this amino acid may be a conditionally

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essential nutrient during catabolic states.[11,12] After abdominal irradiation in rodents, GLN-supplemented feedings decreased toxicity and death.[13] In addition GLN-supplemented parenteral feedings enhance cellularity of the gastrointestinal mucosa and improve immunologic function.[14,15] In preliminary studies patients receiving bone marrow transplants and GLN-supplemented parenteral nutrition demonstrated improved nitrogen retention when compared with matched individuals receiving standard GLN-free intravenous feedings.[16] No deleterious effects of GLN administration were observed. The purpose of this study was to evaluate the effects of GLN-enriched intravenous solutions on body fluid compartments in patients undergoing bone marrow transplantation.

Materials and Methods

Subjects with hematologic malignancies in remission (n = 20) who enrolled in allogeneic BMT protocols in the Brigham and Women's Hospital were studied. After hospital admission patients received central venous catheters, followed by a study of body composition, which was performed 1 week before the BMT (referred to as "initial" measurement, day −7, Fig. 1). The individuals then underwent a program that greatly reduced the endogenous flora of skin and gut, including placement in laminar airflow rooms and oral ingestion of gentamycin, vancomycin, and nystatin. A conditioning regimen was initiated using cytosine arabinoside (3 g/m^2, twice a day for 3 days), cyclophosphamide (1800 mg/m^2, once a day for 2 days), and whole-body irradiation (175 cGy twice a day for 4 days, a total dose of 1400 cGy, n = 18) over a 7-day period. Two additional subjects received only cyclophosphamide (3.6 g/m^2) and busulfan (16 mg/kg). Graft-*versus*-host (GVHD)-prophylaxis consisted of *ex vivo* lymphocyte depletion with ST-1 immunotoxin (Sanofi Research, Inc., Montpelier, France)[17] or a combination of methotrexate and cyclosporin.

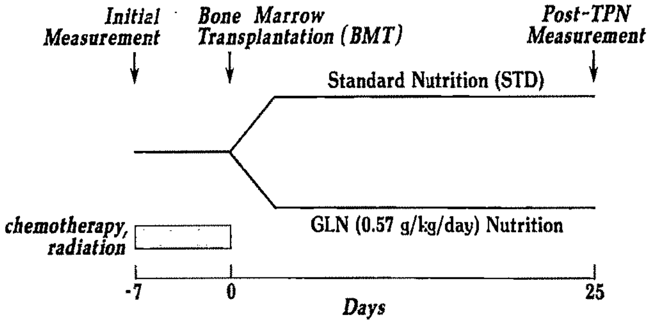


FIG. 1. Study protocol. Initial measurement of body fluid volumes using dilutional and impedance techniques was performed on day −7 followed by a standard dose of chemotherapy and total body radiation administered over the next 7 days. After bone marrow transplantation, patients were randomized to receive either standard parenteral nutrition or GLN-supplemented intravenous feedings. A second measurement of body fluid volumes (Post-TPN) was performed at the termination of TPN (day 25 ± 1).

TABLE 1. *Characteristics of Patient Groups and Healthy Controls (Mean ± SEM)*

Characteristics	STD*	GLN	CTR
N	10	10	10
Age (yr)	33 ± 3	36 ± 3	35 ± 3
Body weight (kg)	69.9 ± 4.5	70.6 ± 3.3	70.0 ± 4.5
Height (cm)	170 ± 2	171 ± 3	172 ± 2
Sex (F/M)	6/4	5/5	3/7
Diagnosis (CML/ AML/misc)	5/4/1	5/4/1	—
GVHD-prophylaxis (ST1/MTX + cyclosporine)	7/3	6/4	—
Splenectomy (≤2 mo)	3	3	—

* No differences were seen between individuals receiving a standard parenteral solution (STD) compared with subjects receiving glutamine-enriched parenteral feedings (GLN) or healthy controls (CTR).

After infusion of allogeneic bone marrow on day 0, patients were randomized by the research pharmacist into those who received standard parenteral nutrition (STD) or those who received GLN-supplemented intravenous feedings (GLN). The control and the experimental groups were matched by balanced design for sex, diagnosis, and GVHD-prophylaxis (Table 1). Patients, investigators, physicians, and nurses were blinded to the randomization.

Total parenteral nutrition was initiated on the day after bone marrow infusion. The caloric requirements of the patients were based on basal energy requirements obtained from standard tables multiplied by 1.50, a calculation previously determined to meet the needs of BMT patients.[18] The protein intake was maintained at 1.5 g/kg/day; nonprotein calories were administered as a 70% glucose and 30% lipid emulsion (Intralipid, Kabi Vitrum, Stockholm, Sweden). Thus the two study solutions were isonitrogenous and isocaloric parenteral solutions. The STD solution contained a commercially available amino acid mixture (Novamine, Kabi Vitrum, Stockholm, Sweden). Crystalline L-glutamine (0.57 g/kg/day, Ajinomoto USA, Raleigh, NC) was added to another commercially available solution rich in essential amino acids (Renamine, Baxter Health Care Corp., McGaw Park, IL)[16] and sterilized by membrane filtration (Millex-GS, 0.22-μm filter, Millipore, Bedford, MA). The patients were allowed an *ad libitum* low-bacterial diet throughout their hospitalization.

The parenteral regimens were terminated when the individuals consumed more than 50% of their energy requirements by the oral route. A second body compositional analysis (referred to as "post-TPN" measurement) was performed immediately at termination of the TPN, and patients were discharged from the hospital shortly thereafter.

Body Compositional Analysis

Volumes of body fluid compartments were assessed by two different methods. The first technique was based on the dilution of bromide and deuterium oxide (heavy water, D_2O).[19] These substances were used as nonradioactive indicators to determine the volume of extracellular (ECW) and total body water (TBW), respectively.

On the morning of study, venous blood samples were obtained to measure background concentrations of these substances. Heavy water was then administered over 1 minute through a centrally placed intravenous line at a dose of approximately 0.15 g/kg body weight (bw); bromide was given over 5 minutes at a dose of 0.75 mL/kg bw. Three hours after injection, venous blood samples were obtained and processed to determine the equilibrium concentrations of these indicators. The heavy water was sterile, pyrogen free, and 99.8% pure (Cambridge Isotope Laboratories, Woburn, MA). The quantity required was measured to the nearest 0.1 mg by determining the difference in weight of a syringe containing this dilutional marker before and after injection. A 3% solution of sodium bromide (wt/vol) was prepared 1 day before the day of study (99.6% pure NaBr, Spectrum, Gardena, CA). The required amount of this 3% solution usually approximated 50 mL and was drawn into 30-cc syringes using standard cold sterilizing techniques (Millex-GS, 0.22-μm Filter, Millipore, Bedford, MA). The weight of the injected NaBr was determined to the nearest milligram.

Bioelectrical impedance analysis was used as a second method of fluid determination. This technique is based on the principle that electrical conduction is proportional to the quantity of water present in a substance. A weak electrical current is passed through the body and is opposed by the body's tissues[20]; this opposition is termed impedance (Ohms, Ω). The impedance signal consists of two components: resistance (R, Ω) and reactance (X_c, Ω). Whole body R is reciprocally related to the volume of TBW,[21,22] whereas the body's X_c may reflect integrity of cellular membranes and associated volume of ECW.[23] A high ratio of X_c to R (X_c/R) is found during health, whereas low values are observed during critical illness.[24] Impedance measurements were performed across the body by attaching electrodes to a hand and a foot as described by others[21] and applying a 50-KHz alternating current generated by a plethysmograph (Model 101A, RJL Systems, Mt. Clemens, MI)

A group of healthy subjects ($n = 10$) served as normal controls for the patients (CTR, Table 1). These individuals were fasted after midnight, and on the study morning venous blood samples were obtained. Heavy water and bromide were taken as a drink from a glass using a straw. The glass was rinsed multiple times with distilled water at a fixed dose of 2 ml/kg bw, and this additional volume was ingested to ensure total administration of the indicators. Equilibrium blood samples were obtained 4 hours later. Subjects were not allowed to eat or drink during the tracer equilibrium period. Body impedance measurements were performed during this period.

Patient Assessment

Vital signs, body weight, and standard blood tests were monitored daily. In addition, the intake of parenteral and enteral nitrogen and calories was calculated daily. Significant clinical events, including infections, were recorded. In all cases clinical infection was associated with fever >38.5°C. Pneumonia was diagnosed by chest-roentgenogram, and bacteremia was associated with positive blood cultures. Soft tissue infection was reflected by clinical signs and symptoms of redness, pain, and local necrosis. Throat and stool were cultured routinely two to three times per week for aerobic bacteria, and additional cultures of blood, urine, sputum, and other sites were obtained when clinically indicated. The number of positive results of all these cultures was calculated between day 0 and the day of intestinal recontamination (mean interval: 25 ± 1 days). The total quantity of blood products (platelets, red blood cells), steroids, and diuretics received was tabulated. Also calculated were the number of nonprophylactic antibiotic agents administered daily and the total number of days of antibiotic use. In addition, the number of hospital days after transplantation was determined for each patient.

Blood Processing

Heparinized blood was centrifuged for 15 minutes at 3000 rpm and the plasma stored in sealed plastic tubes at −20°C. Concentrations of heavy water were determined using mass spectrometry (Model 3/60, Nuclide, Bellefonte, PA).[25] Additional venous blood was allowed to clot for 60 minutes, centrifuged (3000 rpm for 15 minutes), and stored in plastic tubes at −20 C. Before analysis samples again were centrifuged for 4 hours at 3000 rpm using a filter for deproteination (Amicon 30, Amicon Corp., Lexington, MA). Concentrations of bromide were assessed by high-pressure liquid chromatography (HPLC Model LC-6A, Shimadzu Corp., Columbia, MD) using an ultraviolet detector; the technique is a modification of a method used by others.[26] A Partisil SAX-10 column (Whatman Inc., Clifton, NJ) was used at room temperature, and the mobile phase was 25 mmol/L (or less) KH_2PO_4 to assure a retention time for bromide between 17.5 and 19 minutes. The detection wavelength was 195 nm, absorbance was 0.08, and the attenuation was 5. The injection volume

TABLE 2. *Measurements of Body Fluid Compartments (Mean ± SEM)*

Measurement	STD*	GLN	CTR
ECW (L)	14.9 ± 1.0	15.4 ± 0.8	15.2 ± 1.2
TBW (L)	40.3 ± 1.8	41.1 ± 2.4	43.6 ± 2.3
Resistance (R, Ω)	558 ± 26	565 ± 23	523 ± 25
Reactance (Xc, Ω)	67 ± 3	62 ± 3	71 ± 3
Xc/R	0.121 ± 0.004	0.110 ± 0.006	0.137 ± 0.005†

* Volumes of extracellular water (ECW), total body water (TBW), electrical resistance (R), and reactance (Xc) of patients before a standard stress were not different compared with those of healthy controls. However, when the ratio of Xc to R was determined, a significantly smaller value was obtained in both patient groups when compared with controls.

† $p < 0.05$ *vs.* STD, GLN by Fisher's PLSD post-hoc.

was 20 µL, and the samples were analyzed in triplicate. Correlation coefficients for the standard curve were ≥0.998.

Calculations and Definitions

Heavy water dilution space was calculated from the quantity of administered tracer and the venous heavy water concentration after complete distribution.[27] Total body water was calculated from these values by using a correction factor of 1.04, as previously described.[28,29] Extracellular water also was calculated according to the dilution principle, but was corrected for intracellular bromide distribution and the Donnan equilibrium.[30]

Statistical Analysis

The data were analyzed using standard statistical software (StatView #512, Abacus Concepts, Inc., Berkeley, CA) for the Macintosh SE personal computer. Analysis of variance (with Fisher's PLSD *post hoc* test) was used to determine differences between groups at initiation of the study. Linear regression analysis was used to describe the relationship between dependent and independent variables. Paired or unpaired t tests compared parametric data; the Mann-Whitney U tested nonparametric data when appropriate for single comparisons. A chi square and the Fisher's exact test were used to determine differences between expected and observed frequency. A p-value < 0.05 was considered significant. Results are expressed as mean ± standard error of the mean (SEM).

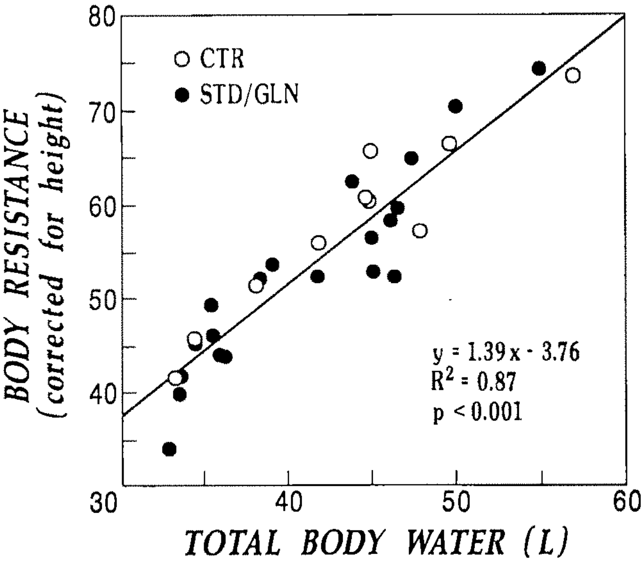


FIG. 2. At baseline, a close relationship was seen between TBW measured by heavy water dilution and body resistance (corrected for height, in cm^2/Ω) in all subjects. Patients and controls were equally distributed along the regression line.

Results

Initial Characteristics of Subjects

The patients groups were similar with respect to age, weight, height, sex, diagnosis, conditioning regimen, or GVHD-prophylaxis (Table 1). Initial ECW, TBW, R, and X_c obtained from all patients were similar and comparable to measurements obtained in healthy controls (NS [not significant] by ANOVA [analysis of variance], Table 2). The ratio of X_c/R obtained in healthy subjects, however, was significantly greater when compared with both patient groups. At baseline an inverse relationship was present between measured TBW and R in all subjects studied ($R^2 = 0.72$, $p < 0.001$). Correcting R for subject's height^2 (in cm^2/Ω) improved this relationship (Fig. 2).[20]

Intravenous Feedings

Both patient groups received comparable amounts of parenteral and enteral calories, parenteral amino acid, and oral nitrogen (Table 3). Likewise both groups of subjects received TPN for a similar duration after BMT (STD: 28 ± 2 *vs.* GLN: 25 ± 2 days, p = 0.25). The period between the initial and the post-TPN body compositional assessment was identical (32 ± 2 *vs.* 32 ± 2 days).

TABLE 3. *Nutrient Intake During Period of Parenteral Nutrition (Mean ± SEM)**

Intake	STD	GLN
Intravenous calories/ day	2330 ± 70	2347 ± 93
Oral calories/day	265 ± 25	379 ± 70
Intravenous nitrogen (g/day)	13.9 ± 0.4	14.5 ± 1.0
Oral nitrogen (g/day)	1.1 ± 0.4	1.5 ± 0.3
Days of administration	28 ± 2	25 ± 2

* Intake of parenteral nutrients was comparable between groups with respect to calories by either route; intravenous and oral nitrogen intake was also similar.

TABLE 4. *Alterations in Body Weight During TPN Administration (Mean ± SEM)*

	Initial	Maximal	Post-TPN	Δ Total	Δ Last 8 days
STD	69.9 ± 4.4	74.4 ± 4.2	71.6 ± 4.4*	+1.8 ± 0.9	−2.8 ± 0.9
GLN	70.6 ± 3.3	74.9 ± 3.0	69.9 ± 3.0*	−0.7 ± 1.0†	−5.0 ± 1.1

Δ Total, difference between the post-TPN and initial body weight measurements; Δ Last 8 days, change in body weight over 8 days preceding the post-TPN determination.

* $p < 0.001$ over time by ANOVA.
† $p < 0.05$ vs. STD by Mann-Whitney U-test.

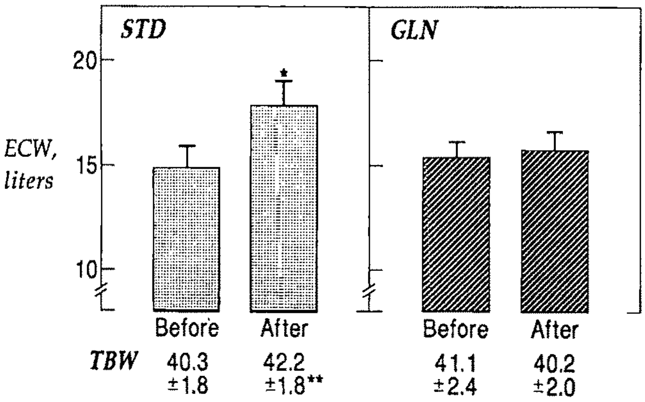
Alterations in Body Weight And Fluid Compartment Volumes

Body weight measured in the STD group was maximal on day 18 ± 1 after BMT and then decreased over the consequent 8 days by 2.8 ± 0.9 kg to 71.6 ± 4.4 ($p < 0.001$ by ANOVA, Table 4). Body weight determined in GLN patients peaked on day 16 ± 2, followed by a more pronounced weight loss of 5.0 ± 1.1 kg. At the post-TPN measurement, STD patients had increased their BW by 1.8 ± 0.9 kg compared with the initial measurement, which was significantly different compared with GLN patients ($p < 0.05$ by Mann-Whitney). With termination of TPN, subjects who had received STD solution retained more than 3 L of ECW (baseline ECW: 18.0 ± 1.1 L *vs.* 14.9 ± 1.0, $p = 0.012$, Fig. 3). This expanded ECW volume was associated with a tendency to increase TBW (42.2 ± 1.8 L *vs.* 40.3 ± 1.8, $p = 0.075$). In contrast patients who received GLN-enriched parenteral nutrition maintained ECW (baseline ECW: 15.8 ± 0.9 L *vs.* 15.4 ± 0.8, $p = 0.49$) and TBW (40.2 ± 2.0 L *vs.* 41.1 ± 2.4, $p = 0.45$).

Alterations in Electrical Properties of the Body

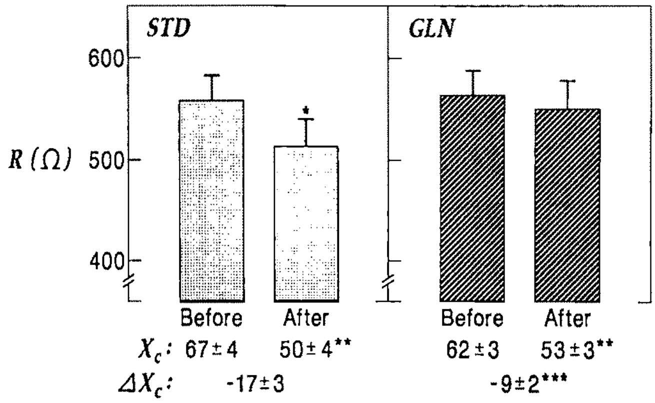
The extracellular fluid retention observed in STD patients was associated with a significant fall in R when compared with baseline values (baseline: 558 ± 26 Ω *vs.* post-TPN: 514 ± 28, $p < 0.05$, Fig. 4). Furthermore X_c was decreased by approximately 25% in these patients ($p < 0.001$). In contrast GLN patients maintained R (565 ± 23 Ω *vs.* 552 ± 27, $p = 0.42$). The fall in X_c was significant but smaller ($p < 0.05$) in GLN patients (62 ± 3 Ω *vs.* 53 ± 3, $p < 0.001$) when compared with the STD patients (67 ± 4 Ω *vs.* 50 ± 4, $p < 0.001$).

High ratios of reactance to resistance (X_c/R) are found in healthy individuals, whereas low values of X_c/R are associated with disease.[24] A significant decrease in X_c/R after BMT occurred in both patient groups combined (initial: 0.116 ± 0.004 *vs.* post-TPN: 0.098 ± 0.004, $p < 0.001$). The $\Delta X_c/R$ was −0.024 ± 0.003 in the STD group, which tended to be greater than the $\Delta X_c/R$ of −0.013 ± 0.005 observed in GLN group ($p = 0.09$). A positive relationship was present between $\Delta X_c/R$ and cumulative dose of steroids administered ($p < 0.02$), total antibiotics used ($p < 0.04$), number of units of packed cells transfused ($p < 0.02$), and days of TPN ($p < 0.04$, Fig. 5). The relationship between length of hospital stay and fall in this ratio approached significance ($p = 0.06$).



* p = 0.012, ** p = 0.075 vs. Before by pairec t-test

FIG. 3. Alterations in ECW and TBW. With termination of TPN (*after*), subjects who received STD solution retained more than 3 L of ECW (18.0 ± 1.1 L *vs.* 14.9 ± 1.0), which was associated with a tendency to increase TBW. In contrast, patients who received GLN-enriched parenteral nutrition showed stable ECW (15.8 ± 0.9 L *vs.* 15.4 ± 0.8 L) and TBW values. Numbers are mean ± SEM. All values in liters.



* p<0.05, ** p<0.001 vs. Before by paired t-test
*** p<0.05 vs. STD by Mann-Whitney

FIG. 4. Alterations in electrical properties of the body. The fluid retention observed in the STD patients was also reflected by a decrease in electrical resistance measured across the body (R: 558 ± 26 Ω *vs.* 514 ± 28, $p < 0.05$). In contrast, resistance in patients who received GLN-enriched TPN was comparable when compared with pretreatment values (R: 565 ± 23 Ω *vs.* 552 ± 27, $p = 0.42$). The decrease in electrical reactance (X_c, in Ω) was significantly attenuated in the GLN patients ($p < 0.05$). Numbers are mean ± SEM.

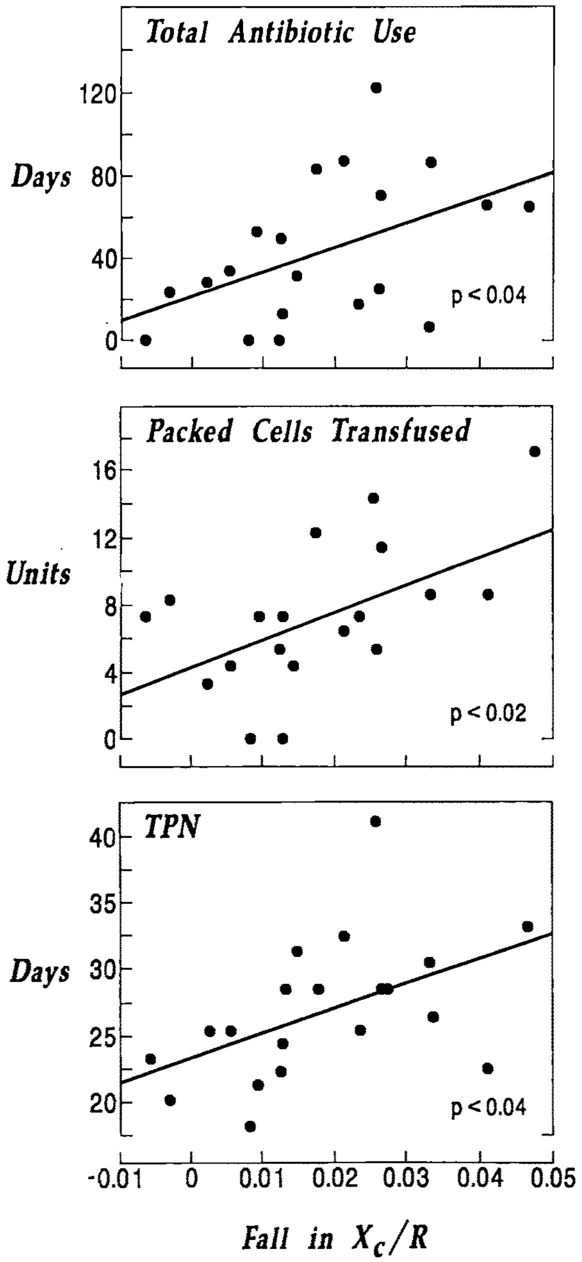


FIG. 5. Associations between decrease in X_c/R ($=\Delta X_c/R$), quantity of antibiotics used, units of red blood cells transfused, and days of TPN received. These associations suggest that this ratio is correlated with the severity of illness.

Treatment Comparability

Daily intravenous and oral fluid intake and electrolytes administered were similar for both groups during the study period. In addition, mean daily fluid losses and fluid balance were comparable between groups (Table 5). No differences were observed between the number of units of blood products (platelets, packed cells) received ($p = 0.96$), or quantity of diuretics ($p = 0.76$) or steroids ($p = 0.65$) administered.

TABLE 5. *Fluid and Medication Administered During TPN (Mean ± SEM)**

Treatment	STD	GLN
Fluid intake (L/day)†	5.553 ± 0.359	5.429 ± 0.278
Fluid output (L/day)†	4.643 ± 0.431	4.557 ± 0.318
Fluid balance (L/day)†	0.911 ± 0.101	0.872 ± 0.152
Packed cells/platelets (units)	15 ± 2	16 ± 3
Lasix (mg prescribed)	106 ± 45	127 ± 51
Steroids (mg cortisol)	168 ± 43	247 ± 152

* No differences were observed between groups with respect to fluid intake, output, and balance; in addition, blood products and medication prescribed were similar.

† Determined over first 18 days of TPN administration, since dropouts after day 18 rendered further comparisons invalid.

Results of Cultures and Clinical Infection

Cultures obtained from the throat, stool, urine, and blood indicated that 45 of 155 in the STD group were positive for the growth of aerobic bacteria (29%, Table 6). In contrast only 29 of 188 cultures (15%) in the GLN group demonstrated bacterial growth (chi square = 9.3, $p < 0.01$). An association was present between increase in ECW and number of positive cultures ($r = 0.47$, $p < 0.05$); the fall in X_c and increase in ECW were also positively correlated ($r = 0.7$, $p < 0.001$).

The occurrence of clinical infections was more frequent in the STD group: Pneumonia was diagnosed in two patients, necrotizing soft tissue infection in two additional individuals, and bacteremia in a fifth patient. In contrast clinical infection was not present in patients receiving GLN ($p = 0.033$ by Fisher's exact test). The mean expansion of ECW in clinically infected STD patients was 5.0 ± 1.4 L, which was significantly more than the mean of 0.7 ± 0.5 L observed in uninfected patients ($p = 0.007$ by Mann-Whitney, Fig. 6); alterations in uninfected STD patients were not different from values determined in GLN subjects (1.2 ± 0.8 *vs.* 0.4 ± 0.5, NS).

Discussion

The purpose of this study was to examine the effects of GLN-enriched parenteral feedings on distribution of body fluids in a homogenous group of patients undergoing

TABLE 6. *Cultures and Clinical Infection*

	STD	GLN
Positive	45	29
Total	155	188
%	29	15*
Patients with clinical infection	5/10	0/10†

* $p < 0.01$ *vs.* STD by chi square.

† $p < 0.05$ *vs.* STD by Fisher's exact test.

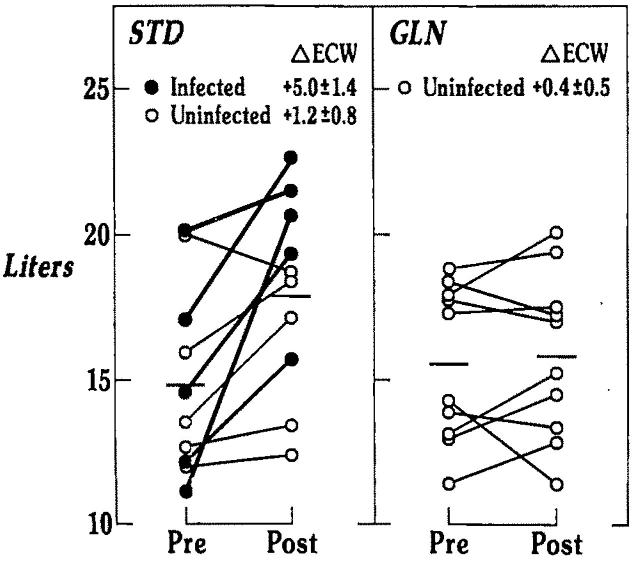


FIG. 6. The relationship between ECW expansion and infection. A clinical infection (pneumonia, soft-tissue infection, or bacteremia) was present in half of the patients who received the STD solution (●); these infected patients had expanded their ECW by 5.0 ± 1.4 L. The remainder of the STD patients did not show infection (O), and Δ ECW in these patients was significantly lower (+1.2 ± 0.8 L). In contrast, infection in patients receiving the GLN solution was absent, whereas ECW volumes were stable (+0.4 ± 0.5 L).

a standard catabolic insult. Our studies confirm previous reports that the administration of commercially available intravenous feedings is associated with expansion of ECW in a similar patient population.[10] In our study patients receiving the STD-TPN retained more than 3 L of ECW, which also was reflected by a marked fall in the electrical resistance of the body. In contrast matched subjects who received the GLN-enriched solution demonstrated normal ECW volumes and stable electrical resistance at the termination of the TPN. Because the two patient groups received similar quantities of sodium, water, calories, protein, blood products, diuretics, and steroids, exogenous or treatment factors did not appear to contribute to this effect.

Glutamine may affect fluid balance by multiple mechanisms. For example GLN is taken up by the kidney and donates NH_3, which couples with H^+ to form urinary NH_4^+. Under physiologic conditions 30% to 50% of the daily acid load is excreted by this mechanism.[31] Excretion of H^+ is coupled with mandatory reabsorption of Na^+ and associated water. Patients receiving the STD-TPN demonstrated a higher frequency of positive bacterial cultures and clinical infection, which may be associated with increased generation of acid substances. If this occurred the increased H^+-load may have enhanced Na-reabsorption and contributed to the subsequent ECW expansion in the STD patients. A positive correlation between increased ECW and number of positive cultures ($p < 0.05$) and the greater expansion of ECW in clinically infected patients are both suggestive of this mechanism. Venous HCO_3^- was not different between groups (STD: 24 ± 1 vs GLN: 23 ± 1), however, and urinary sodium excretion measured in selected patients also was comparable.

An alternative explanation may be that GLN ameliorated the increase in capillary permeability, which typically occurs after chemotherapy, irradiation, or severe infection. Injury to endothelial cells may be the result of increased production of free oxygen radicals, and GLN may be important in providing antioxidant protection. For example GLN appears to be an essential precursor in the synthesis of glutathione (GSH).[32] During specific conditions GSH is a major antioxidant that protects tissues from free oxygen radical damage.[33,34] Glutathione depletion may occur in the STD patients, and GSH may have been maintained in patients receiving GLN. Hinshaw et al.[35] suggested an additional mechanism of protection associated with GLN. They found that GLN added to culture media protected endothelial cells after H_2O_2 exposure by serving as a fuel for adenosine triphosphate generation.[35] Others have reported that GLN suppresses inflammation and the associated edema formation that occur after various inflammatory stimuli.[36] Glutamine therefore may diminish extravasation of fluids by preventing "leakage" through endothelial cells and subsequently may blunt the expansion of the extracellular volume. Body weight gain was similar for both groups, however, over the first 16 to 18 days after BMT. Similar increases in body weight may indicate that a comparable expansion of the extracellular fluid compartment occurred in both groups, and that after these events GLN-supplemented nutrition facilitated a more efficient diuresis associated with recovery. Such effects suggest that GLN may be associated with modulating the neuroendocrine control of sodium and water homeostasis.[37]

The effects of GLN on elaboration of arginine vasopressin, renin, or aldosterone are unknown, although GLN is thought to have effects on the central nervous system.[38] Furthermore GLN may stimulate increased elaboration of glucagon.[39] Animal studies have shown an association between portal infusion of glucagon and increased glomerular filtration rate.[40] Previous studies in normal volunteers, however, did not show elevations of glucagon in peripheral blood samples after intravenous administration of GLN[41]; concentrations of glucagon were not measured in our patients.

Restoration of extracellular fluid volumes also may be related to the beneficial effects of GLN on gut metabolism. Glutamine attenuates atrophy of gut mucosa, which is associated with prolonged administration of intravenous feedings,[14] and this amino acid serves as an important fuel for enterocytes, colonocytes, macrophages, and lymphocytes. Increased availability of GLN during the regeneration phase of the mucosa after gastrointestinal injury[9] may have facilitated repair and accelerated mobilization of bowel and mesenteric edema. Moreover, GLN administration may have facilitated lymphocyte proliferation and enhanced phagocytosis, as demonstrated by others.[42] The combined responses of mucosal repair and immunoenhancement may have improved host defense and attenuated microbial invasion and subsequent infection, which frequently originates from the gastrointestinal tract in this patient population.[43]

Before treatment the body fluid compartments were similar in patients and controls: these groups also demonstrated comparable body resistance (R) and reactance (X_c). The ratio of X_c to R, however, was significantly lower in patients compared with normals. Several observations have suggested a relationship between a low X_c/R and the presence of illness. For instance, progressive malnutrition in acquired immune deficiency syndrome (AIDS) patients was associated with a declining ratio.[44] In addition another quantative measure of malnutrition also suggests that X_c falls with nutritional depletion in surgical patients.[45] We also have observed that this ratio falls markedly in surgical patients[24] and returns to normal with recovery. In the present study, the fall in the ratio was generally related to increased numbers of therapeutic interventions such as the dose of steroids and antibiotics administered, units of blood products transfused, days of TPN administration, and length of hospital stay. These associations suggest that the X_c/R ratio may be an objective measure of severity of illness and possibly may predict recovery.

In conclusion, catabolic patients receiving a standard glutamine-free intravenous solution after intensive chemotherapy and total body irradiation demonstrated expanded extracellular fluid compartments coupled with low values of electrical resistance; these events were associated with a greater number of positive microbial cultures and more clinical infection. In contrast matched patients who received GLN-enriched intravenous feedings demonstrated unaltered fluid compartments and a stable body resistance; number of positive cultures was diminished and clinical infection was absent in GLN patients. Intravenous feedings supplemented with GLN aid in restoring normal body fluid distribution after critical illness, possibly by attenuating microbial invasion and infection of the host.

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REFERENCES

1. Wilkinson AW, Billing BH, Nagy G, et al. Excretion of choride and sodium after surgical operations. Lancet 1949; 1:640–644.
2. Moore FD, Ball MR, eds. Metabolic response to surgery. Springfield, IL: Charles C. Thomas, 1952.
3. Dudley HF, Boling EA, Lequesne LP, et al. Studies on antidiuresis in surgery: effects of anesthesia and posterior pituitary antidiuretic hormone on water metabolism in man. Ann Surg 1954; 140: 354–367.
4. Llaurado JG. Increased excretion of aldosterone immediately after operation. Lancet 1955; 1:1295–1298.
5. Flear CT, Bhattacharya SS, Singh CM. Solute and water exchanges between cells and extracellular fluids in health and disturbances after trauma. J Parenter Enter Nutr 1980; 4:98–120.
6. Halmagyi DFJ, Broell J, Gump FE, et al. Hypermetabolism in sepsis: correlation with fever and tachycardia. Surgery 1974; 75:763–770.
7. Böck JC, Barker BC, Clinton AG, et al. Post-traumatic changes in, and effect of colloid osmotic pressure on the distribution of total body water. Ann Surg 1989; 210:395–405.
8. Moore FD, Olesen KN, McMurray JD, et al, eds. The Body Cell Mass and Its Supporting Environment. Body Composition in Health and Disease. Philadelphia-London: WB Saunders, 1963.
9. MacDonald GB, Shulman SH, Sullivan KM, et al. Intestinal and hepatic complications of human bone marrow transplantation. Part I and II. Gastroenterology 1986; 90:460–477, 774–784.
10. Cheney CL, Gittere Abson K, Aker SN, et al. Body composition changes in marrow transplant recipients receiving total parenteral nutrition. Cancer 1987; 59:1515–1519.
11. Stehle P, Mertes N, Puchstein C, et al. Effect of parenteral glutamine peptide supplements on muscle glutamine loss and nitrogen balance after major surgery. Lancet 1989; 1:231–233.
12. Lacey JM, Wilmore DW. Is glutamine a conditionally essential amino acid? Nutr Rev 1990; 48:297–309.
13. Klimberg VS, Salloum RM, Kasper M, et al. Oral glutamine accelerates healing of the small intestine and improves outcome after whole abdominal radiation. Arch Surg 1990; 125:1040–1045.
14. O'Dwyer ST, Smith RJ, Hwang TS, et al. Maintenance of small bowel mucosa with glutamine-enriched parenteral nutrition. J Parenter Enter Nutr 1989; 13:579–585.
15. Burke DJ, Alverdy JC, Aoys E, et al. Glutamine-supplemented total parenteral nutrition improves gut immune function. Arch Surg 1989; 124:1396–1399.
16. Ziegler TR, Benfell K, Smith RJ, et al. Safety and metabolic effects of L-glutamine administration in humans. J Parenter Enter Nutr 1990; 14(Suppl):137S–146S.
17. Antin JH, Bierer BE Smith BR. Depletion of bone marrow lymphocytes with an anti-CD-5 monoclonal immunotoxin (ST-1 immunotoxin). Prog Clin Biol Res 1990; 333:207–215.
18. Szeluga DJ, Stuart RK, Brookmeyer R, et al. Energy requirements of parenterally fed bone marrow transplant recipients. J Parenter Enter Nutr 1985; 9:139–143.
19. Schloerb PR, Friis-Hansen BJ, Edelman IS, et al. The measurement of total body water in the human subject by deuterium oxide dilution. J Clin Invest 1950; 29:1296–1310.
20. Hoffer EC, Meador CK, Simpson DC. Correlation of whole-body impedance with total body water volume. J Applied Physiol 1969; 27:531–534.
21. Lukaski HC, Johnson PE, Bolunchuk WW, et al. Assessment of fat-free mass using bioelectrical impedance measurements of the human body. Am J Clin Nutr 1985; 41:810–817.
22. Kushner RF, Schoeller DA. Estimation of total body water by bioelectrical impedance analysis. Am J Clin Nutr 1986; 44:417–424.
23. Scheltinga MR, Kimbrough TD, Jacobs DO, et al. Altered cellular membrane function can be characterized by measuring body reactance. Surg Forum 1990; 41:43–44.
24. Scheltinga MR, Jacobs DO, Kimbrough TD, et al. Identifying body fluid distribution by measuring electrical impedance. Submitted for publication.
25. Wong WW, Lee LS, Klein PD. Deuterium and oxygen-18 measurements on microliter samples of urine, plasma, saliva and human milk. Am J Clin Nutr 1987; 45:905–913.
26. Miller ME, Cosgriff JM, Forbes GB. Bromide space determination using anion-exchange chromatography for measurement of bromide. Am J Clin Nutr 1989; 50:168–171.

27. Schoeller DA, Van Santen E, Peterson DW, et al. Total body water measurements in humans with ^{18}O and ^{2}H labeled water. Am J Clin Nutr 1980; 33:2686–2693.
28. Culebras JM, Moore FD. Total body water and the exchangeable hydrogen: I. Theoretical calculation of nonaqueous exchangeable hydrogen in man. Am J Physiol: Regulatory Integrative Comp Physiol 1977; 1:R 54–59.
29. Schoeller DA, Kushner RF, Taylor P, et al. Measurement of total body water: isotope dilution techniques. *In* Roche AF, ed. Body composition assessments in youths and adults. Williamsburg, VA: Ross Laboratories, 1985, pp 24–29.
30. Bell EF, Ziegler EE, Forbes GB. Letter to the editor. Pediatr Res 1984; 18:392–393.
31. Rose BD. Clinical physiology of acid-base and electrolyte disorders. New York: McGraw-Hill, 1977, pp 189–210.
32. Miller A, Horiuchi M. In vitro incorporation of radioactive amino acid precursors in the GSH of human erythrocytes. J Lab Clin Med 1963; 60:756–764.
33. Stein HJ, Hinder RA, Oosthuizen MMJ. Gastric mucosal injury caused by hemorrhagic shock and reperfusion: protective role of the antioxidant glutathione. Surgery 1990; 108:467–474.
34. Hong RW, Helton WS, Rounds JD, et al. Glutamine-supplemented TPN preserves hepatic glutathione and improves survival following chemotherapy. Surg Forum 1990; 41:9–11.
35. Hinshaw DB, Burger JM. Protective effect of glutamine on endothelial cell ATP in oxidant injury. J Surg Res 1989; 49:222–227.
36. Jain P, Khanna NK. Evaluation of anti-inflammatory properties of l-glutamine. Agents Actions 1981; 11:243–249.
37. van Gelder NM. Brain taurine content as a function of cerebral metabolic rate: osmotic regulation of glucose derived water production. Neurochem Res 1989; 14:495–497.
38. Cocchi R. Antidepressive properties of L-glutamine. Acta Psychiatr Belg 1976; 76:658–666.
39. Li S, Nussbaum NS, McFadden DW, et al. Addition of L-glutamine to total parenteral nutrition and its effects on portal insulin and glucagon and the development of hepatic steatosis in rats. J Surg Res 1990; 48:421–426.
40. Lang E, Tschernko E, Schulze E, et al. Hepatic regulation of renal function. J Am Soc Nephrol 1990; 1:66 (Abstract No 34P).
41. Lowe DK, Benfell K, Smith RJ, et al. Safety of glutamine-enriched parenteral nutrient solutions in humans. Am J Clin Nutr 1990; 52:1101–1106.
42. Parry-Billings M, Evans J, Calder PC, et al. Does glutamine contribute to immunosuppression after major burns? Lancet 1990; 336:523–525.
43. Navari NM, Buckner CD, Clift RA, et al. Prophylaxis of infection in patients with aplastic anemia receiving allogeneic marrow transplants. Am J Med 1984; 76:564–572.
44. Fischer H, Ott M, Lembcke B, et al. Bioelektrische Impedanz Analyse (BIA): eine Methode zur Messung des Ernärungszustandes bei Patienten mit HIV infektion unterschiedlicher Stadien. 3. Deutscher Aids-Kongress, Hamburg, November 1990 (Abstract).
45. McDougall D, Shizgal HM. Body composition measurements from whole body resistance and reactance. Surg Forum 1986; 37:42–44.

DISCUSSION

DR. EDWARD M. COPELAND, III (Gainesville, Florida): There is little question that glutamine is metabolically beneficial during critical illness. For example endotoxin, tumor necrosis factor, and interleukin-1 (IL-1) all stimulate glutamine uptake by endothelial cells threefold. Perhaps the endothelial cell requirement for glutamine during critical illness is increased to support metabolism and repair, as shown by both Dr. Wilmore's laboratory and by ours.

Likewise Tom Austgen and Chip Souba from our laboratory have shown that preinfusion of glutamine to rats decreases the amount of pulmonary edema that develops after standard endotoxin challenge.

Dr. Scheltinga has investigated the effect of glutamine on maintenance of water homeostasis in a group of young, nutritionally stable patients who are challenged with systemically toxic doses of chemotherapy and with whole-body irradiation. These patients received an average of 5½ L fluid daily for 3 to 4 weeks, along with Lasix, platelets, blood, and steroids. Both groups of patients gained an equal amount of weight for the first 16 days, but the glutamine group returned to baseline by day 25. The standard TPN group appeared sicker, because they had more infections and a lower ratio of reactance to resistance, which correlated with the use of steroids, antibiotics, and platelets.

Dr. Scheltinga, I must ask you, with all of these variables in this patient population, how do you know that glutamine was an effective compound, or was the standard therapy group just more ill and the return to baseline sooner in the glutamine group the consequent expected outcome?

You must have other measures of nutritional state, such as serum albumin concentration, retinol-binding protein, etc., that demonstrate better metabolic maintenance in the glutamine group. Share these with us.

Why did the two groups receive different amino acid solutions, particularly one high in essential amino acids? Did not this add another confounding variable?

I want to believe, and in fact do believe, that glutamine ameliorates capillary fragility, lowers infection rate, provides antioxidant protection, and repairs endothelial cell injury. However it is difficult for me to extrapolate these conclusions solely from your study of change in body fluid compartments and in bioelectrical impedance in this patient population.

DR. JOHN R. BORDER (Buffalo, New York): This is an extremely important paper. It is important not because of the fluid balance changes in the abstract but because those fluid balance changes reflect septic complications. The radiation and chemotherapy delivered to these patients effectively destroy their gut mucosa. To prevent septic complications, the patient and his gut are largely sterilized before radiation and chemotherapy and the patient is thereafter maintained in isolation with as much sterility as possible. During this time there is severe diarrhea and emesis plus the isolation procedures that make many standard study techniques practically impossible. The electrical impedance technique was used here as a practical study technique.

During the period of isolation, not only is there regrowth of gut mucosa but also of the gut bacterial population. The septic problems reported here are almost undoubtedly gut-origin septic states. The essence of the paper is that glutamine-enriched amino acid solutions as compared with standard amino acid solutions greatly reduced the gut-origin septic state problems. This is to be expected granted the importance of glutamine as a gut mucosa energetic fuel.

It must be clearly noted in these patients that the critical maneuver after destruction of the gut mucosa is to achieve regrowth of the gut mucosa before the bacteria repopulate the gut. This requires both gut mucosa energetic fuels and amino acids. Glutamine is one such energetic fuel. The ketone bodies and short-chain fatty acids are others, and these are clearly of most benefit to the colonic gut mucosa. These agents can now easily be given in quantity as their mono or diglyceride ester. The *in vivo* esterases then release the ketone body as short-chain fatty acid and glycerol.

This paper confirms in a practical clinical way the existence of the gut origin septic states and suggests that support of these unfortunate young people can be greatly improved. However because the gut-origin septic states are probably very common in all critically ill people, the information obtained may have a much broader application then in the patients studied.

DR. BASIL A. PRUITT, JR (San Antonio, Texas): This report is yet another in the series of studies from Dr. Wilmore's laboratory demonstrating that hormonal manipulation and the addition of specific dietary constituents can influence the risk of infection, preserve lean body mass, and alter the duration and velocity of convalescence in surgical patients.

Dr. Scheltinga has presented data that extend this group's earlier observations and show that glutamine added to the intravenous nutrients of patients who had received an allogeneic bone marrow infusion after high-dose chemotherapy and total body irradiation prevented the expansion of the extracellular fluid compartment associated with standard total parenteral nutrition and was associated with a lesser number of positive cultures.

Those findings are of potentially broad surgical application, because infection remains the most common cause of morbidity and death in critically ill surgical patients. Of particular interest are the data indicating that bioelectrical impedance measurements, which are noninvasive, can be used to detect changes in the ratio of reactance to resistance and appear to reflect severity of illness and may predict recovery.

There are several questions, the answers to which may help us in evaluating the authors' findings. The data in Table 3 indicate that the glutamine patients received almost 3000 more calories and 25 more grams of nitrogen during the 25-day study period. I ask whether those differences could have influenced the extracellular fluid (ECF) differences?

Table 4 indicates that the post-TPN body weight increased by 1.8 kilos in the standard total parenteral nutrition patients, and decreased by 0.7 kilos in the glutamine patients. However Table 5 shows that daily fluid balance favored the standard group by almost a full liter over the 25-day study period. If one subtracts that from the post-TPN body weight change, does the difference in weight change lose significance?

Several of the measurement techniques can be influenced by variables other than nutrient supply, and it will be necessary to know (1) whether protein and other dissolved solids were removed from the plasma samples in which deuterium dilution was measured, and (2) whether bromine space was corrected for body mass index, because patients with lower body mass index and less fat have greater ECF volumes per unit body mass.

If one corrects your ECF measurements for body fat content, do the differences between groups change?

The use of bioelectrical impedance in situations with disturbed water distribution between intracellular and extracellular water has been criticized by some. Because others have reported that glutamine induces hepatocyte swelling, do the differences in the reactance to resistance ratios simply reflect a difference in the distribution of water across the cellular membrane in the glutamine group? That would certainly be consistent with the comparability of the observed alterations in body weight.

Lastly because cultures were obtained, both routinely and when clinically indicated, does the difference in positive culture simply reflect the frequent reculturing of infected patients? In fact do the changes in body fluid compartments merely reflect the aggregate effect of infection, because in the STD group 5 of the 10 patients had a clinically diagnosed infection, whereas none of the glutamine patients had such infections?

I compliment the essayists again on a very provocative study that further defines the optimum nutritional support regimen for a class of critically ill patients.

DR. EDWIN A. DEITCH (Shreveport, Louisiana): I rise to make a comment, ask the authors two questions, as well as to compliment them on a very interesting study carried out in an extremely complicated patient population. One explanation for your findings is that glutamine is not directly attenuating fluid expansion and edema but, instead, is preventing infection and thereby indirectly affecting fluid retention. That is both the patients receiving standard TPN and those receiving glutamine-supplemented TPN appeared to have equally expanded extracellular volumes after the initial 14 to 17 days of the study. Subsequently what seemed to be occurring was that the glutamine-fed patients were able to diuresis the extra fluid they had retained, whereas some of the patients on standard TPN could not because they were infected.

Consequently I have several questions for the authors about this point. First could you identify where the fluid was sequestered and were there any associations between the sites of fluid sequestration, such as the lung, and the sites of infection, in other words, pneumonia? Secondly because glutamine is essential for optimal leukocyte or intestinal cell proliferation, do you have any information on the state of immune function or intestinal integrity in the patients? Because these bone marrow recipients received cytotoxic therapy, including radiation and chemotherapy, it is quite likely that the patients receiving standard TPN could have developed a relative deficiency of glutamine that subsequently predisposed to the development of systemic infection or intestinal barrier leak?

DR. DONALD S. GANN (Baltimore, Maryland): I think this is a very important study, and I think Dr. Scheltinga, Dr. Wilmore, and their colleagues have shown us that there is at least a way to prevent infection in this group undergoing very hazardous treatment. Nothing that I am going to say should interfere with that evaluation.

However it seems to me that there are some problems relating to their analysis of the handling of fluids here that I simply cannot make add up. I find it impossible to understand how subjects can gain 2 kg of weight and 2 L water without changing their excretion of salt and water. And I believe that that must not be the case. If it is not the case, then I would like to know what were the observed changes in sodium and a water excretion?

Dr. Scheltinga mentioned in his talk the possible implication of vasopressin and aldosterone. Those substances can limit excretion, but they cannot, without changes of intake, account for an increase in body content. They can only account for a decrease in loss.

We heard from other discussants that the intakes of the two groups were not constant, apart from glutamine. Were they constant with respect to electrolyte, or were there variations in electrolyte as well as in amino acids?

Furthermore it appears to me that the group given the standard treatment is clearly not homogeneous. I wonder if the authors could separate for us the responses in the infected *versus* the noninfected group. Did the noninfected group in fact behave as did the standard group, or was the treatment itself possibly involved in eliciting a hormonal response? It seems to me that the response that the authors have observed is probably almost exclusively related to the infection, at least as I saw the data.

DR. FRANCIS D. MOORE (Boston, Massachusetts): I would like to congratulate Dr. Wilmore on another beautifully done, beautifully designed, beautifully conceived and analyzed study. I would like also to congratulate Dr. Schloerb for keeping the heavy water, nonradioactive method of total body water not only alive but refining it immensely since his early days of working on it with us at the Brighain so many years ago.

I would hope that he would do the same thing for the total body potassium method, which needs modernizing. This would enable him to measure the body cell mass (BCM) directly in such patients, using either some stable isotopes or tiny doses of radioactivity, which is now possible, a thousandth of the amount we used to use. If we had body cell mass measurements on these patients, we would answer some of the questions that I know are in Dr. Wilmore's mind and the minds of many of the discussants.

I would like to point out that the "lean body mass" (LBM) includes bone and fascia, and it includes other extracellular solids that are not fat, so they are called "lean." The LBM is much heavier than the BCM and includes lot of inactive stuff ("chassis") as well as the metabolizing cells ("engine"). What we really need to measure is the body engine, the mass of cells in the body that burn fuel and do work; this is the BCM, and that can be measured by potassium dilution.

Thirdly, I would like to emphasize that we are not looking here at surgical trauma. I would like to reflect a moment on a philosophical point. In this meeting and in many other surgical meetings, surgical trauma is somehow regarded as the master switch that turns on a variety of changes. How many papers on burns have we heard, in which it is just assumed that these changes are typical of burns or unique to burns? Well of course they are not. Burns are just very complicated illnesses, and such injuries cause such changes.

Here we have radiobiologic injury. That is the injury these patients suffered. It is not "surgical trauma." It is massive, however, with loss of body cell mass and, of course, total wipeout of immunocompetence. There is a demonstrable excretion of the intracellular products of the cells that are specifically damaged by the radiation. At these doses these cells are largely in the hemopoietic system, but, as shown years ago, you can measure decay products in the urine, and you can estimate how many grams or kilograms of cells have been wiped out.

Although this is *not* surgical trauma, it is of immense interest. Think of all the massive radiotherapy that has been done in this country, and still we know almost nothing about the metabolic response to that sort of injury. So this is an important paper.

As to the bone marrow transplant itself, all that is desired is typing a few cells. These people have not reconstituted their immunocompetence or their entire bone marrow in 25 days! But the transplant therapy can be started because then they can get out of isolation, and can go back to eating.

Now what does glutamine do, if anything? Is it a diuretic of some sort? Although I have not seen this manuscript, I would be very interested to know about the urea and total nitrogen excretion in the two groups. Why is it that the water balance seems so much better? I join Dr. Pruitt and Dr. Gann in saying that I cannot make any mathematical sense out of it. If you gain 3.8 L of ECW, you generally gain 3.8 kg of weight, unless you've lost 3.8 kg of BCM, which is not only unbelievable, but not demonstrated here.

So I need to see this manuscript and look at the numbers to understand what is going on there.

It is also stated that this glutamine is "good" for infection. Now in these patients, everything known to man (and a few things only known to women) are used to prevent infection. They are all on multiple antibiotics the whole time. Some of them are physically isolated in the "life island" kind of environment. They are the most vulnerable patients that we have, because radiation has wiped out their immunity.

So that to take one molecule, glutamine, in rather small doses, and say that it is responsible for the difference of infection rate, seems to me to be epistemologically unsupportable.

Then I think we almost always tend to overlook the problem of whether these patients are synthesizing body cells in any part of their body, and I would like to know what sort of exercise regimens they were on and what sort of body-cell–maintaining programs were done before during and after the treatment?

I would like to congratulate the authors. A good paper at the American Surgical is one that arouses a lot of discussion. So thanks a lot.

DR. MARC SCHELTINGA (Closing discussion): Thank you very much for these kind remarks. Dr. Copeland asked whether the magnitude of changes in nutritional state was comparable between the two study groups. At baseline patients had a similar albumin and comparable ideal body weight. Alterations in these and other parameters such as triceps skinfold, mid-arm circumference, and grip strength were similar during the study period. We do not think that the observed changes in body composition could be explained by differences in the contents of essential *versus* nonessential amino acids in the study solutions. The control individuals actually received more essential amino acids (45%) than the experimental group (33%), and this should favor the control group. The experimental group received approximately 0.57 g glutamine per kilogram per day, which amounts to approximately 40 g/day. These diets were well tolerated, and disproportionate increases in ammonia or glutamic acid were not observed in the glutamine group. No changes in mental status occurred.

Dr. Border, thank you for your kind comments. We believe as you do that further development must be done to improve nutritional care of our patients.

Dr. Pruitt asked whether the differences in fluid and calorie intake could explain the changes in extracellular water. These intakes were quite similar. Our patients were not studied in a clinical research center setting, however, but on a ward that contained laminar airflow rooms. Many of these patients developed diarrhea, which complicated precise balance measurements. Therefore body compositional measurements become more useful to evaluate changes in body hydration.

Dr. Gann asked whether differences in extracellular water were reflected by differences in fluid balance. Our analysis over the first 18 days of study indicated that no such differences in fluid intake, output, and balance were present between groups. In addition similar quantities of blood products, steroids, and diuretics were received by the subjects during this time. Comparison between groups beyond 18 days of TPN administration was hampered because all patients did not require intravenous nutrition and some were being discharged from the hospital. The increase in body weight over the first 18 days after transplantation was comparable between groups, however, which may indicate similar rates of fluid accumulation. After this period patients receiving the control solution maintained their body weight. In contrast to the control patients, subjects receiving the glutamine solution were able to mobilize their retained fluid, and this resulted in a loss of body weight over the last 8 days of study. This diuresis was associated with the absence of clinical infection. Because of the association between infection and fluid retention in the control group, we are continuing to assess this patient population to determine if glutamine enrichment of intravenous feedings may prevent infection.

Dr. Deitch inquired as to whether the differences in water might be accounted for by fluid sequestration in the gastrointestinal tract. We do not know the answer to this question, but are exploring this issue by using regional bioelectrical impedance measurements. This approach should help us identify the site of the extra fluid.

Dr. Moore noted that these patients did not undergo surgical trauma, but radiobiologic injury. This group of patients is ideal to study because they can be evaluated before they undergo a standard stress, and the extent of cellular injury is comparable and of significant duration. Moreover, they have marked disruption of their intestinal mucosa, exhibit variable degrees of sepsis, and also require parenteral nutrition for 3 to 4 weeks. Our control patients exhibited rates of infection similar to other reported series, and we are encouraged by the decrease in morbidity and attenuated stress response observed in the patients receiving glutamine. Clearly, more patients must be studied, and we continue prospective, double-blinded trials in this group of individuals to verify the potential advantages of nutrients and growth factors in facilitating recovery.

In conclusion, if one studies animals under carefully controlled conditions, the efficacy of glutamine-enriched solutions is becoming increasingly evident. Such novel therapies will only be meaningful, however, if one demonstrates beneficial effects in critically ill patients. In this group of bone marrow transplant patients, conditions were optimal to perform a controlled prospective, blinded trial. This study provides findings that suggest glutamine-enriched solutions may be beneficial in this treatment setting.

Thank you for the opportunity to present these data.

Effect of Colectomy on Bile Composition, Cholesterol Crystal Formation, and Gallstones in Patients With Ulcerative Colitis

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Bile composition and the presence of cholesterol crystals in bile were studied in bile samples obtained at the time of surgery in patients with ulcerative colitis. Some patients were sampled before colectomy and others at a second operation months to years after a colectomy. Patients in the precolectomy group were found to have bile composition similar to control patients without gallstones, and few had crystals in their bile. In the postcolectomy group, cholesterol concentrations were very high, all biles were supersaturated, and almost all patients had cholesterol crystals in their bile. These findings are similar to those found in persons with cholesterol gallstones. Twenty patients have been followed for at least 3 years after colectomy and to date three have formed cholesterol gallstones.

THE CRYSTAL OBSERVATION time (or nucleation time) is a test used to determine the rapidity of cholesterol crystal formation in bile. Bile from patients with cholesterol gallstones usually exhibits rapid crystallization in 1 to 2 days.[1,2] Most biles from patients without cholesterol gallstones have longer crystal observation times, but occasionally rapid nucleation is seen in the absence of gallstones. Recently we reported a group of such patients without gallstones, but with rapidly nucleating bile.[3] We noted that a number of these patients previously had had a colectomy for ulcerative colitis. Furthermore two patients under our care formed gallstones after colon surgery for ulcerative colitis. Formation of gallstones after colectomy in ulcerative colitis has been described.[4]

The purpose of this study was to determine whether ulcerative colitis or colectomy in patients with this disease produced changes in bile composition that predisposed these patients to crystal formation and perhaps ultimately to gallstones. To do this gallbladder bile composition and crystal observation time of patients with ulcerative colitis were examined in samples obtained at the time of surgery. Patients then were followed to determine if any formed gallstones.

Methods

Bile Collection

Gallbladder bile was collected from 28 patients during elective surgery for ulcerative colitis. Seventeen patients were admitted for elective surgery and had no previous surgery on the digestive tract. At surgery 11 had colectomy, 4 had colectomy and pelvic pouch construction, and 2 had colectomy and Kock pouch construction. This group is referred to as the precolectomy group. A postcolectomy group consisted of 11 patients who had had subtotal colectomy 5 months to 14 years previously; six of these had colectomy between 5 months and 1 year before sampling, and five had the procedure more than 3 years before sampling. At the time of admission, 2 of the 11 had a Kock pouch, one had a pelvic pouch, and the remainder had conventional ileostomy. Eight of the eleven were admitted for conversion from conventional ileostomy to pelvic pouch (7 patients) or Kock pouch (1 patient), two for revision of Kock pouch, and one for excision of a pelvic pouch. Only very small lengths of ileum, (1–4 cm) were resected in any patient. Preoperative ultrasound examinations were done in all 28 patients, and none had cholelithiasis.

Bile samples were obtained at the start of surgery. The technique of bile sampling for normal gallbladders recently has been described in detail.[5] Briefly a 22-gauge needle was advanced under the gallbladder serosa for 1

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cm before puncturing the mucosa to enter the gallbladder. This is done to create a self-sealing tunnel in the gallbladder wall. The bile was aspirated as completely as possible using gentle gallbladder compression to facilitate this. Procedures were approved by the Human Ethics Committee of the University of Toronto. Written informed consent was obtained before bile collection.

Follow-up Studies

Twenty of twenty-eight patients (9 men and 11 women) were examined by abdominal ultrasound from September 1990 to January 1991 at least 36 months after bile sampling. The others either could not be contacted or they refused.

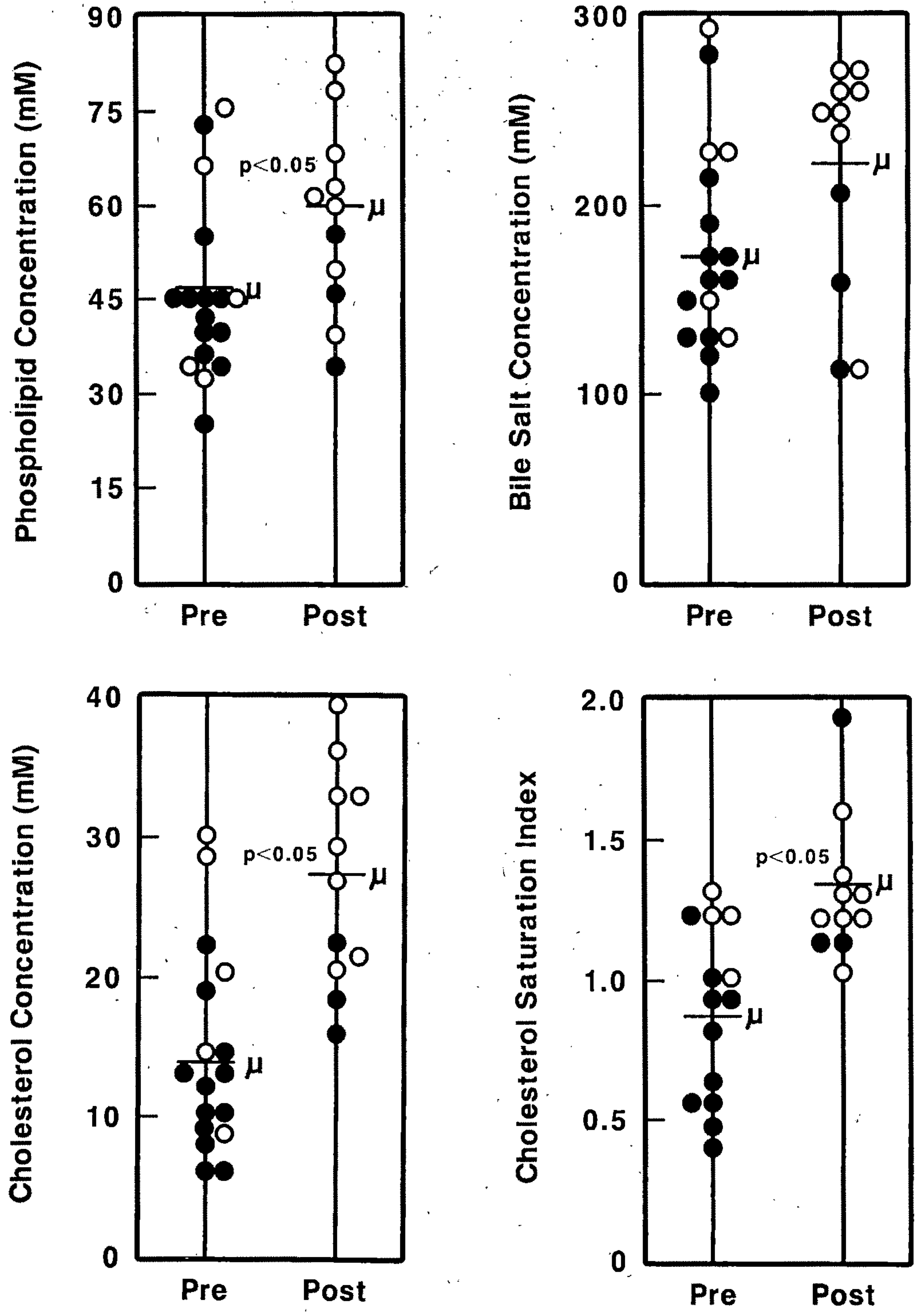

FIG. 1. Bile salt, phospholipid and cholesterol concentrations, and CSI in patients with ulcerative colitis. The precolectomy group in each comparison is shown on the left. Results for postcolectomy patients are shown on the right. Open symbols indicate that the particular sample contained cholesterol crystals.

Crystal Observation Time

The term "crystal observation time" is used instead of "nucleation time," because the assay measures both nucleation and stone growth.[5] Crystal observation time, defined as the time of appearance of cholesterol crystals (in days) in a bile sample that has been initially cleared of crystals, was determined using a modification[2] of the technique of Holan et al.[1]

Chemical Methods

Cholesterol was determined by gas liquid chromatography,[6] phospholipid by inorganic phosphate by Bartlett's technique,[7] and bile salts by the 3-hydroxysteroid dehydrogenase method.[8] Cholesterol saturation index (CSI) was calculated using Carey's critical tables.[9] Total biliary protein concentration was measured as previously described by a fluorometric assay.[10]

Statistics

The results of the two groups (precolectomy and postcolectomy) were compared using Student's t test or chi square test where appropriate. Only one patient was sampled twice, in other words, before and after colectomy at two separate operations, and the data have been treated as unpaired.

Results

There were no significant differences in the mean ages or weights of patients in the precolectomy and postcolectomy groups (36.9 ± 11.2 [standard deviation (SD)] years and 35.4 ± 10.2 [SD] years, respectively, and 65.0 ± 14.9 kg and 60.7 ± 8.6 kg, respectively) at the time that their bile was sampled. There were relatively more males in the precolectomy group (9/17) than in the postcolectomy group (4/11). This difference was not significant (chi square test).

The concentrations of individual biliary lipids and the CSI are given in Figure 1. The results for total lipid concentration, total protein concentration, and crystal observation time are presented in Figure 2. The mean cholesterol concentration in the postcolectomy group was almost double that in the precolectomy group, 28 mmol/L *versus* 14 mmol/L. This difference was highly significant, ($p < 0.01$). Phospholipid and bile salt concentrations also were greater in the postcolectomy group, but the differences were less remarkable than that for cholesterol. The difference in phospholipid concentration was significant ($p < 0.01$), but that for bile salt was not ($p > 0.05$ and < 0.10). The total lipid concentration, in other words, the sum of concentrations of the three lipids, was significantly greater in the postcolectomy group ($p < 0.05$).

Concentration ratios for cholesterol-bile salt, cholesterol-phospholipid, bile salt-phospholipid, and cholesterol-total lipid were calculated for each sample. Means and standard deviations of these values are given in Table 1. The means of the cholesterol-bile salt, cholesterol-phospholipid, and cholesterol-total lipid concentration ratios were significantly greater in the postcolectomy group, but there was no difference in the bile salt-phospholipid ratio. These comparisons of ratios confirm that the difference

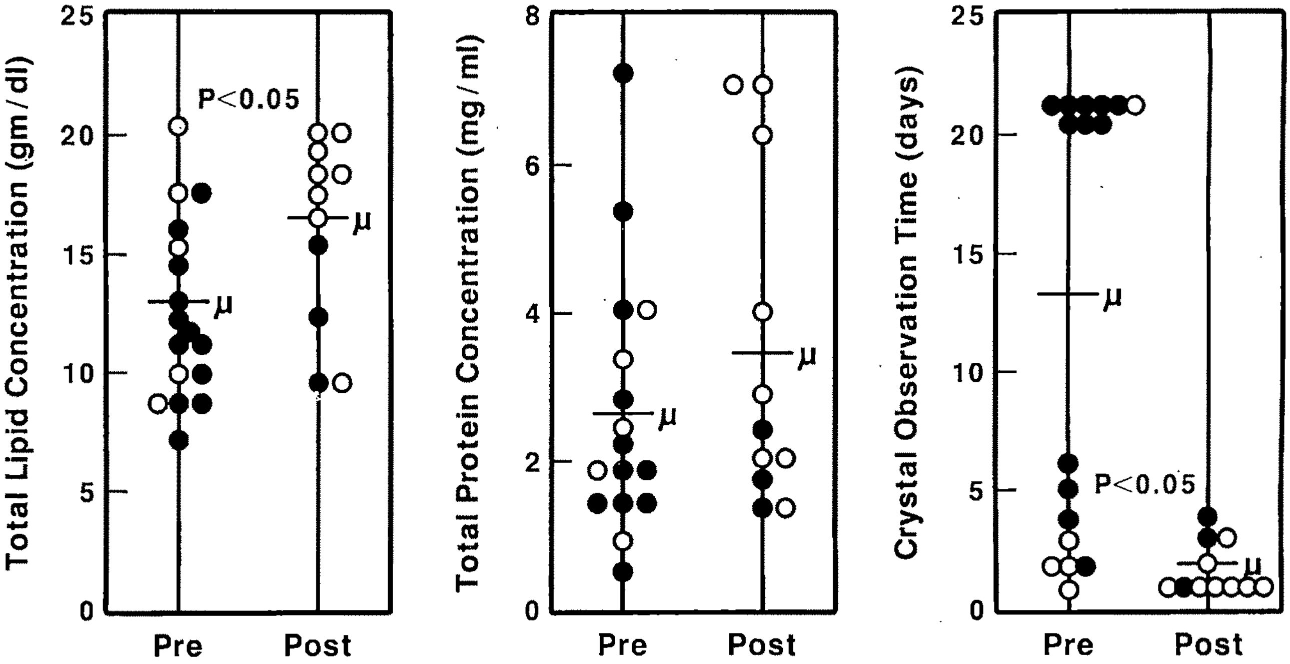

FIG. 2. Total lipid concentration, total protein concentration, and crystal observation time in the two patient groups. Symbols as for Fig. 1.

TABLE 1. *Concentration Ratios of Biliary Lipids in Precolectomy and Postcolectomy Groups*

Ratio	Precolectomy (Mean ± SD) $\times 10^2$	Postcolectomy (Mean ± SD) $\times 10^2$
Cholesterol:phospholipid	31.4 ± 11.4	46.8 ± 4.2*
Cholesterol:bile salt	8.0 ± 2.8	12.7 ± 2.8*
Cholesterol:total lipid	7.0 ± 2.6	10.9 ± 3.6*
Phospholipid:bile salt	26.1 ± 10.0	27.1 ± 4.6

* Significantly different from precolectomy group ($p < 0.01$).

in cholesterol concentration between the two groups is proportionately greater than the differences in phospholipid or bile salt concentrations between groups.

The cholesterol saturation index, crystal observation time, and prevalence of crystals in bile of patients in the precolectomy group were generally similar to previously presented groups of control patients.[1-3] The mean CSI in the precolectomy group was 0.88; only 5 of 17 (29%) had rapid crystal observation times of 3 days or less, and crystals were present in 5 of 17 (29%) patients. In contrast results in the postcolectomy patients were much more like those of persons with cholesterol gallstones.[1-3] In the postcolectomy group, all biles were supersaturated, with a mean CSI of 1.32. The crystal observation time was 3 days or less in 10 of 11 patients (91%), and 8 of 11 (73%) had cholesterol crystals in bile. In one patient gallbladder bile was sampled at two surgeries 7 months apart. A subtotal colectomy was performed at the first operation, and a pelvic pouch at the second procedure. In this patient the CSI increased from 0.94 to 1.13 and the crystal observation time fell from 21 days to 1 day. Three of six patients whose colectomy was performed in the year before sampling had crystals in their bile, but all five patients colectomized more than 3 years before sampling had crystals. Thus it appears that supersaturation appears early after surgery, and crystalobilia follows at a variable time, but is present in all patients by 3 years. Total protein concentration was not significantly different in the two groups.

Twenty of twenty-eight patients (9 men and 11 women) were examined by ultrasound from September 1990 to January 1991 at least 36 months after bile sampling. Of course all were in the postcolectomy state at this time. Computing the time of follow-up from the time of colectomy, the time of follow-up ranges from 3 to 19 years in these 20 patients, with a median of 4.8 years. Three patients have developed multiple gallstones as visualized on ultrasound. A 36-year-old woman in the "precolectomy" group was found to have gallstones 7 years after colectomy, pelvic pouch construction, and the time when her bile was sampled. A 47-year-old woman in the "postcolectomy" group had stones demonstrated 4 years after colectomy and Kock pouch construction, which was 3 years after the nipple valve was revised and the time that her sample was obtained. The third patient to develop stones is a 37-year-old man in the "postcolectomy group" whose ultrasound was positive 7 years after colectomy and 5 years after pelvic pouch construction, when his bile was sampled. All three patients are asymptomatic with respect to their stones.

Discussion

This study is somewhat unusual in that patients were studied before and after a specific intervention causing lithogenic bile, and then were followed for several years until, at least in a few patients, gallstones appeared. The original findings of the study are that there is a remarkable difference in the bile composition and crystal observation time between patients with ulcerative colitis that have had colectomy and those that have not. Those with colectomy have findings characteristic of patients with cholesterol gallstones.[1-3] Conversely bile obtained before colectomy is similar to that of other control patients without stones.

The observed changes are not transient but persist for years after colectomy. It appears from our results that colectomy in ulcerative colitis patients first leads to a period of supersaturation without crystals. This is followed by the appearance of crystals, and then culminates in the development of gallstones in some patients after several years.

The postcolectomy patients were healthy, nutritionally fit, and were admitted for an elective operation. Most were on no medication. Although it might be tempting to conclude that the difference between groups was due to the presence of active ulcerative colitis, in actuality it was the precolectomy group that had normal bile composition and nucleation time, and the postcolectomy group that had the abnormal results. It would therefore appear that colectomy itself is important in the genesis of these findings.

Why might colectomy in this group of patients result in supersaturated rapidly nucleating bile? A simple answer might be that such patients lose increased amounts of bile salts once an ileostomy is formed. Patients with ileal disease or resection lose more bile salts and thus are predisposed to supersaturated bile and gallstones. The most common clinical situation in which this occurs is ileal Crohn's disease. This does not explain the findings of our study, however. We previously showed that persons with conventional ileostomy or a Kock pouch do not have abnormally large bile salt loss. Their fecal bile salt excretion rates range from 350 to 500 mg/day.[11] Only patients with substantial ileal resections had higher than normal losses of about 1100 mg/day.[11] None of the patients in the present study had ileal resection of greater than 4 cm.

Hylander et al[12] noted daily losses of 1.2 mmoles in patients with conventional ileostomy (about 480 mg) and 1.8 mmoles (about 725 mg) in patients with a Kock reservoir. The upper end of the range in six normals in their study was 1.7 mmoles per day. The slight differences in values are most likely due to variations in methodology. Bile salts were directly measured by gas-liquid chromatography (GLC) in the earlier study[11] and an indirect method using reduction of nicotinamide-adenine dinucleotide (NAD) was used in the later investigation.[12] Modest overestimations might have occurred because of the presence of other NAD-reducing substances in stool.[12] In both studies the bile salt losses were within or very close to reported normal values, however,[11] and could not account for reduced bile salt secretion rates and bile salt concentrations in bile. Thus supersaturation should not be produced by bile salt losses of this magnitude. This is further substantiated by the fact that there is considerable reserve bile salt synthetic capacity in the liver, such that bile salt secretion rates do not fall until fecal losses rise severalfold. For instance in the rhesus monkey losses five times the norm may occur without alteration in secretion rates.[13]

Another decisive argument against bile salt loss as the cause of our findings is that bile composition has been examined in animal models of ileal resection[14,15] and in patients with ileal dysfunction or resection.[16,17] In the studies of Bickerstaff and Moosa[14] and Pitt et al.,[15] ileal resection in prairie dogs caused a decrease in biliary lipid concentrations, in other words, just the opposite of that observed in this study. Studies in man are more difficult to compare because absolute bile salt concentrations were not reported.[16,17] Farkkila[16] actually found a decrease in CSI in patients with ileal resection in whom fecal bile salt losses were several times normal. Conversely Dowling et al.[17] found an increase in the CSI in patients with resection or ileal diseases. Previous studies in the primate from this laboratory have demonstrated that moderate bile salt losses actually improve the CSI.[18] The lost bile salts are made up by synthesis from cholesterol, which is thereby diverted from biliary secretion.[18] It is only when fecal bile salt losses exceed the bile salt synthetic capacity that the bile salt secretion rates fall and CSI rises.[18] Presumably Farkkila[16] and Dowling et al.[17] examined patients with different degrees of bile salt malabsorption. Therefore it takes large increases in fecal bile salt loss to cause increases in the CSI, and such increases do not occur in patients who have had colectomy even when a pouch is constructed, provided there has been no ileal resection.

Our findings indicate a large increase in the lipid concentration, especially cholesterol concentration in bile. These observations must be explained. There are two events that can increase the concentration of a solute in bile, namely increased net solute input or decreased net water input into bile. Shifts in water balance occur throughout the biliary tree and gallbladder. The gallbladder acts to concentrate bile solutes by removing 80% of the water in hepatic bile. One possible contributing factor to increased lipid concentration of gallbladder bile might be reduced gallbladder motility in the postcolectomy period. This might result in stasis, increased time for reabsorption of water, and increased concentration of solutes. This cannot fully explain our findings, however. If a change in water balance affecting concentration of bile lipids were the explanation, one would expect the same relative effect on all lipids. Also one would assume that protein concentrations would be affected in the same way as lipids, and an increase in protein concentration would be anticipated. In this study a disproportionate increase in cholesterol concentration occurred that must be explained by an alteration in cholesterol secretion into or absorption from bile. Cholesterol absorption from the gallbladder may occur in animals[19] but it seems unlikely that colectomy would result in a selective increase in this function. The results seem to point to increased cholesterol secretion into bile. Control of cholesterol secretion into bile is complex and can be affected by cholesterol delivery to the liver, cholesterol synthesis rates, and the degree to which hepatic cholesterol is routed either to bile or peripheral tissues. To understand the cause of the findings in the postcolectomy patients, cholesterol secretion rates must be directly measured, and cholesterol balance studies must be performed.

This study has focused on patients with ulcerative colitis. It would be desirable to examine postcolectomy patients who do not have ulcerative colitis, because it is possible that colectomy alone causes the changes described. Unfortunately there are few opportunities for obtaining gallbladder bile from colectomized patients with diagnoses other than ulcerative colitis, as they rarely have second surgical procedures. Bile-rich duodenal aspirates can be used to measure CSI, but are of no use in obtaining absolute concentrations of lipids, and the use of such material in measuring the crystal observation time is dubious. An animal model of colectomy may be a useful experimental approach.

Three of the twenty patients we have followed for a minimum of 3 years have developed gallstones. It is not possible to be completely certain yet whether this represents an increase over what would be expected. From reported series one would expect gallstones to have developed in about 7% (1 in 20) in these patients.[20,21] It also must be remembered that our patients were shown not to have gallstones at the time of surgery and developed them in an interval of 3 to 7 years after a negative ultrasound. The chances of doing this in a population of the age studied is in the order of 3% to 4%. At this time, however, the numbers are too small to be certain that an unusually high incidence of stones follows colectomy. Further follow-up is needed to obtain clear data. In a previous study, 23.2% of patients with ulcerative colitis developed gallstones after colectomy.[4] In this study patients with Crohn's disease and ulcerative colitis were followed. The ulcerative colitis patients were not reported separately. Their age and length of follow-up was not given. It is not certain that an increased rate of gallstone formation was present, but this seems likely. Such studies will also be of practical importance, because if it is shown that a substantial proportion of these patients do form cholesterol gallstones, then preventive measures such as a daily nighttime dose of ursodeoxycholic acid may be indicated. The present results also demonstrate that although crystalobilia is almost inevitable in colectomized ulcerative colitis patients, gallstone formation takes years to occur and may perhaps never happen. Once again this points out that there are multiple requirements for stone formation.

In summary patients with ulcerative colitis who have colectomy develop supersaturated bile soon after surgery, and after some time crystals appear in their bile. A proportion of these patients go on to form cholesterol gallstones. The mechanism of the supersaturation is unknown, but the likeliest cause is an increase in cholesterol secretion into bile. Further studies are required to clarify the pathogenesis and to determine whether prophylaxis is required in this group of patients.

References

1. Holan KR, Holzbach RT, Hermann RE, et al. Nucleation time: a key factor in the pathogenesis of cholesterol gallstone disease. Gastroenterology 1979; 77:611–617.
2. Gollish SH, Burnstein MJ, Ilson RG, et al. Nucleation of cholesterol monohydrate crystals from hepatic and gall-bladder bile of patients with cholesterol gall stones. Gut 1983; 24:836–844.
3. Strasberg SM, Toth JL, Gallinger S, et al. High protein and total lipid concentration are associated with reduced metastability of bile in an early stage of cholesterol gallstone formation. Gastroenterology 1990; 98:739–746.
4. Kurchin A, Ray JE, Bluth EI, et al. Cholelithiasis in ileostomy patients. Dis Colon Rectum 1984; 27:585–588.
5. Strasberg SM, Harvey PRC, Hofmann AF. Bile sampling, processing and analysis in clinical studies. Hepatology 1990; 12:176–182.
6. Morin RJ, Elms NJ. Rapid microanalysis of cholesterol in bile and serum by gas chromatography. Ann Clin Lab Sci 1975; 5:52–56.
7. Bartlett GR. Phosphorous assay in column chromatography. J Biol Chem 1959; 234:466–468.
8. Talalay P. Enzymatic analysis of steroid hormones. Methods Biochem Anal 1960; 8:119–143.
9. Carey MC. Critical tables for calculating the cholesterol saturation of native bile. J Lipid Res 1978; 19:945–955.
10. Harvey PRC, Upadhya GA, Toth JL, Strasberg SM. Fluorometric assay of protein in native human bile. Clin Chim Acta 1989; 183: 147–154.
11. Kay RM, Cohen Z, Siu KP, et al. Ileal excretion and bacterial modification of bile acids and cholesterol in patients with continent ileostomy. Gut 1979; 21:128–132.
12. Hylander E, Ladfoged K, Lykkegaard N, et al. Excretion, deconjugation and absorption of bile acids after colectomy for ulcerative colitis. Scand J Gastroenterol 1986; 21:1137–1143.
13. Dowling RH, Mack E, Small DM. Effects of controlled interruption of the enterohepatic circulation of bile salt secretion, synthesis and pool size in the Rhesus monkey. J Clin Invest 1970; 49:232
14. Bickerstaff KI and Moosa AR. Effects of resection or bypass of the distal ileum on the lithogenicity of bile. Am J Surg 1983; 145: 34–40.
15. Pitt HA, Lewinski MA, Muller EI, et al. Ileal reasection-induced gallstones: Altered bilirubin or cholesterol metabolism. Surgery 1984; 96:154–162.
16. Farkkila MA. Biliary cholesterol and lithogenicity of bile in patients after ileal resection. Surgery 1988; 104:18–25.
17. Dowling RH, Bell GD, White J. Lithogenic bile in patients with ileal dysfunction. Gut 1972; 13:415–420.
18. Strasberg SM, Petrunka CN, Ilson RG. Effect of bile acid synthesis rate on cholesterol secretion rate in the steady state. Gastroenterology 1976; 71:1067–1070.
19. Neiderhiser DH, Harmon CK, Roth HP. Absorption of cholesterol by the gallbladder. J Lipid Res 1976; 17:117–124.
20. Giunchi G. Findings on gallstone disease in the ISTAT investigation. *In* Capocaccia L, Ricci G, Angelico F, et al, eds. Epidemiology and Prevention of Gallstone Disease. Boston: MTP Press, 1984, pp 5–8.
21. Ricci G and the GREPCO Group. The GREPCO group programmes: aims and prevalence data. *In* Capocaccia L, Ricci G, Angelico F, et al, eds. Epidemiology and Prevention of Gallstone Disease. Boston, MTP Press, 1984, pp 9–14.

DISCUSSION

DR. HENRY A. PITT (Baltimore, Maryland): For some time it has been known that patients who have undergone ileal resection or who have ileal Crohn's disease are at increased risk for gallstone formation. It has not previously been appreciated, however, that patients with ulcerative colitis are at increased risk to form gallstones after colectomy.

We have been studying the pathogenesis of gallstone formation after ileal resection. Many of these patients also are receiving long-term TPN, and gallbladder stasis is clearly a factor leading to stone formation in these patients. When we first began looking at this phenomenon, we thought that we were dealing with a problem of cholesterol metabolism. As we examined this problem further, however, we found that cholesterol metabolism was reasonably normal, that gallbladder stasis was important, and that biliary calcium and bilirubin metabolism were altered. Moreover both animals and patients undergoing small bowel resection were found to have pigment gallstones. Therefore I would like to ask the authors whether they have any information on biliary calcium and bilirubin levels or about the types of stones that form after colectomy in patients with ulcerative colitis.

You have shown that the cholesterol saturation index goes up, that the cholesterol concentration of gallbladder bile increases relatively more than the other lipids, and that cholesterol crystal appearance time decreases after colectomy. The real question raised by your study is why colectomy causes these changes. Two possibilities that you did not mention are increased intestinal absorption or altered hepatic processing of cholesterol. Do you have any data on these aspects of cholesterol metabolism in your postcolectomy patients? With respect to cholesterol crystal appearance time, you have been one of the leaders in demonstrating that biliary proteins are important in this process. Do you have any data on biliary proteins, total protein, mucin, or nonmucin glycoproteins in these patients before or after colectomy?

Finally, and perhaps most importantly, are your precolectomy and postcolectomy groups really comparable? You have studied one group of patients before colectomy and another group of patients at the time of pouch construction. Are these two groups of patients similar with respect to age, sex, weight, and nutritional status? Is it possible that some of these patients with ulcerative colitis coming to colectomy were nutritionally depleted, had been NPO for prolonged periods, or had been on TPN, all factors that would alter cholesterol metabolism? In comparison were the patients coming to elective pouch construction months or years after colectomy in a better nutritional state?

DR. STEVEN M. STRASBERG (Closing discussion): Thank you, Henry. We do not know the type of stones that have formed, because these patients are asymptomatic, and their gallstones have not been removed. We assume, given that they have high cholesterol saturation index and cholesterol crystals in bile, that they have cholesterol gallstones.

We have not yet studied the alterations in cholesterol metabolism leading to these stones. Perhaps one of the more important points in this study is that colon resection leads to alterations in cholesterol metabolism. We might be able to learn something about intermediary cholesterol metabolism by examining this type of a model in animals.

We have not measured mucous glycoproteins and we have not subfractionated the proteins.

The question about whether the groups are really comparable is an important question. I would like to point out the following. The precolectomy patients were individuals who came in for elective colectomy. In general they were in good health but they did have ulcerative colitis. These individuals had the normal bile. It was the people in the postcolectomy group who had the abnormal bile. They were admitted for an elective surgery quite remote from the time of their colectomy for ulcerative colitis and were in very good health. In other words the abnormal bile was not the result of the presence of a diseased colon.

Evaluation and Surgical Treatment of Severe Chronic Constipation

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Patients with chronic constipation may have one of several physiologic disorders, not all of which are amenable to operative therapy. The aim of this study was to test colonic and pelvic floor function preoperatively, to identify patients suitable for surgery based on these studies, and to determine operative outcome over time. Between 1987 and January 1991, 277 patients referred for severe symptoms of chronic intractable constipation underwent colon transit studies, measurement of anal canal pressures and reflexes, and measurements of anorectal angle movements and efficiency of evacuation. Balloon expulsion studies, electromyography of the pelvic floor, and defecating proctograms also were done. Based on these studies, patients were categorized as having: slow transit constipation (STC), 29 patients; pelvic floor dysfunction (PFD), 37 patients; STC + PFD, combined slow transit and pelvic floor dysfunction, 14 patients; and irritable bowel syndrome (IBS), 197 patients. Slow transit constipation patients underwent abdominal colectomy and reanastomosis. Pelvic floor dysfunction patients underwent pelvic floor retraining only. Patients with STC + PFD underwent pelvic floor retraining followed by abdominal colectomy. Irritable bowel syndrome patients were treated symptomatically. Among the 38 patients operated on (STC and STC + PFD), there was no operative mortality. Prolonged ileus developed in 13%, and small bowel obstruction occurred in 11% of patients. On follow-up, a mean of 20 months after ileorectostomy, no patient was constipated, none required a laxative, and none was incontinent. The mean number of stools per day was four. The authors concluded that a prospective evaluation of colonic and pelvic floor function reliably delineated constipated patients with slow transit, suitable for operative management, from those with pure pelvic floor dysfunction or irritable bowel syndrome, who were not. Abdominal colectomy and ileorectostomy in the slow transit patients was safe and effective, resulting in prompt and prolonged relief of constipation.

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CONSTIPATION IS A symptom of a complex condition that results from different pathologic processes. The term constipation implies not only infrequent defecation but also difficult defecation. Perhaps the best general definition is that of Drossman et al.'s, "Two or fewer stools per week and/or straining at stool more than 25% of the time."[1] Extracolonic causes for constipation are legion[2] and need to be excluded primarily. The colonic causes are either structurally or functionally based, with the latter being further divided into constipation caused by colonic dysmotility[3] or disordered defecation.[4-7]

To achieve predictable success in managing constipated patients, it is important that underlying pathophysiologies are identified objectively; in this way patients amenable to aggressive surgical or medical intervention can be identified. We therefore developed an evaluation strategy to categorize constipated patients on the basis of physiologic tests, bearing in mind that documenting a physiologic abnormality may not necessarily mean that surgery is indicated. Indeed with a disorder such as constipation, one with multiple causes and possibly complicated by psychomotor overtones, the results of surgery, particularly abdominal colectomy and ileorectostomy, are unpredictable at best.[8-12]

Our evaluation strategy (Fig. 1) aimed to determine the cause of colonic constipation using quantitative tests of colonic, rectal, and anal canal function. The hypothesis was that patients could be evaluated accurately and placed into the appropriate pathophysiologic category such that only patients suitable for surgery would be operated on, with improved results and a predictable outcome. Our aim was to evaluate patients referred for severe constipation systematically to first select operative candidates and then to determine operative outcome over time.

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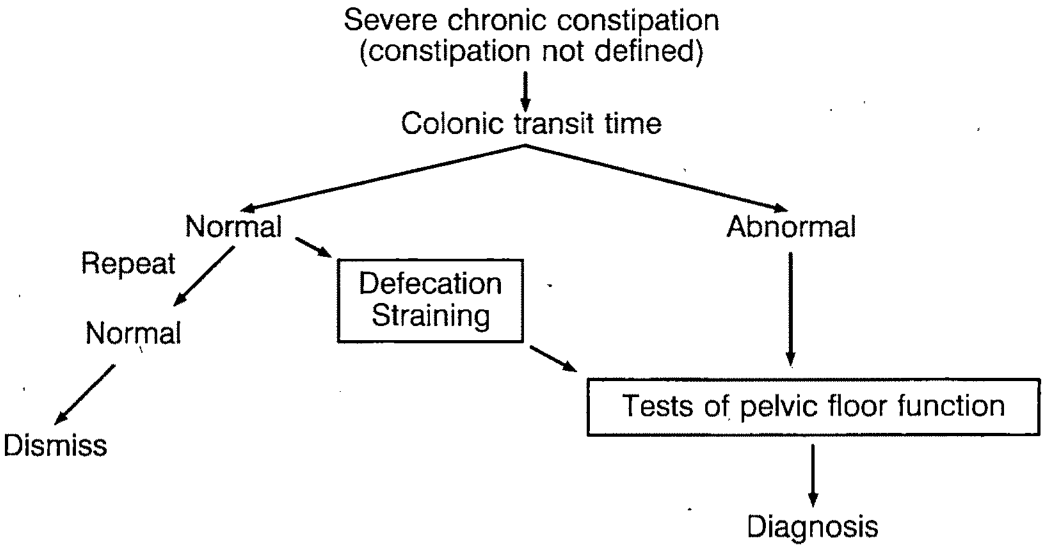
Severe chronic constipation
(constipation not defined)
Colonic transit time
Normal
Abnormal
Repeat
Normal
Dismiss
Defecation Straining
Tests of pelvic floor function
Diagnosis

FIG. 1. Diagram of evaluation scheme used to categorize intractably constipated patients into diagnostic and therapeutic groups.

Patients and Methods

Between 1987 and 1990, 277 patients were referred for symptoms of severe constipation. Only patients with a protracted, chronic—sometimes lifelong—history of constipation and those who were deemed not amenable to further medical management by their referring physicians were assessed. Patients with recent onset of constipation were specifically not evaluated. Moreover physical examination and an initial series of tests, including barium enema or colonoscopy, had failed to uncover a contributing abnormality in all patients. Specifically patients had an anatomically normal colon; patients with megacolon, megarectum, volvulus, prolapse, evidence of colonic pseudo-obstruction, tumor, or polyp were excluded.

Patients studied underwent a series of tests of colonic and pelvic floor function that had been validated previously.

Physiologic Tests

Colonic Transit Test. Transit of solids through the colon was measured using a technique validated by Metcalf and others.[13] Transit through the different segments of the colon (right, left, rectosigmoid) also was determined. Mean colonic transit among 73 controls was 36 ± 4 hours. The upper limit of normal was 72 hours (2 standard deviations above the mean). Patients with transit times longer than 72 hours therefore were deemed to have slow colonic transit.

Pelvic Floor Function. The next step in the evaluation was to perform objective tests of pelvic floor function to quantitate defecation efficiency.

Anorectal Manometry. Perfused four-channel manometry determined resting and squeeze anal canal pressures, the presence of the rectal anal sphincter inhibition response, and compliance of the rectal wall.[14] High sphincter pressures have been associated with functional disturbances in some patients[15]; an absent rectal anal sphincter inhibitory response implies loss of ganglion cells (Hirschsprung's disease); and a highly compliant rectum implies impending megarectum.

Electromyography. Concentric needle electromyography (EMG) was performed to determine the electromyographic characteristics of the puborectal muscle and the external anal sphincter in response to squeeze and defecation straining. The normal response to defecation straining is silencing of the electrical activity and concomitant relaxation of the muscles. Some patients with defecation disorders have a characteristic paradoxical increase or no change in the motor activity of these muscles while straining,[16,17] and the muscles do not relax.

Scintigraphic Balloon Topography. This study defines movements of the anorectal angle and pelvic floor using scintigraphic techniques with low radiation exposure.[18] In previous preliminary studies,[19] among controls the anorectal angle opened a mean of 17 ± 3°, whereas in patients with defecation disorders the change was only 4 ± 4° ($p < 0.05$). Moreover in controls the perineum descended a mean of 2.3 ± 0.2 cm with straining, compared with 0.5 ± 0.1 cm in patients with disordered defecation ($p < 0.05$).

Scintigraphic Evacuation. The efficiency of defecation was quantified by measuring the amount of artificial radiolabeled stool evacuated from the rectum.[20] In preliminary studies[19] among healthy volunteers, the mean (± standard deviation [SD]) percent of stool evacuated in 10 seconds was 80 ± 3%, whereas in patients with defecation abnormalities it was 34 ± 6% ($p < 0.05$).

Balloon Expulsion. A test of integrated pelvic floor function, balloon expulsion, was introduced by Preston et al.[21] We used a similar method. A balloon attached to a catheter was inserted into the rectum and inflated with 50 mL warm water. Subjects then attempted to pass the balloon spontaneously. If spontaneous evacuation did not occur, weight was added incrementally to the catheter until the balloon could be passed. In preliminary studies[19] we found that most control subjects could spontaneously evacuate the balloon. In controls who could not spontaneously pass the balloon, the mean amount of weight required to pass the balloon was 126 ± 41 g. Patients with defecation disorders, however, could not spontaneously pass the balloon, and the mean (± SD) amount of weight required to facilitate passage was 590 ± 114 g ($p < 0.05$).

Defecating Proctogram. This study, described by Mahieu and others,[22] documents the anatomy of the rectum and anal canal during straining. Among patients with difficult defecation, occult rectal prolapse and physiologically significant rectoceles are visualized readily. It is imperative that the studies be interpreted with caution, however, because some degree of intussusception is demonstrated in nearly half of healthy young volunteers.[23]

This series of tests of pelvic floor function was performed, because no individual study has been a reliable discriminator pathognomonic for pelvic floor dysfunction.

Upper Gastrointestinal Manometry. Multichannel perfused catheter studies have been described previously.[24] Patients were candidates for study if symptoms of upper gastrointestinal distress were present. These included nausea, vomiting, and bloating within 30 minutes of eating, weight loss, and upper abdominal pain.

Diagnostic Categories

Patients were placed into the following four diagnostic groups based on results of the function studies.

I. STC; slow transit constipation—in these patients, colon transit was abnormally slow and pelvic floor function normal.
II. PFD; pelvic floor dysfunction—these patients had normal colon transit but abnormal pelvic floor function.
III. STC + PFD; slow transit constipation and pelvic floor dysfunction—these patients had abnormally slow transit and abnormal pelvic floor function.
IV. IBS; irritable bowel syndrome—these patients had normal colon transit and normal pelvic floor function. They therefore had no quantifiable abnormality of transit or pelvic floor function and, for lack of a more precise term, these patients were diagnosed as irritable bowel syndrome.

Results

Patient Characteristics

Table 1 details the demographic and symptomatic data on the 277 patients by diagnostic category.

Patients. The largest group of patients was the one with normal parameters of bowel function (IBS). Importantly only 83 of 277 patients (30%) had objective evidence of either colon or pelvic floor dysfunction.

Age and Sex. Patients with either pure pelvic floor dysfunction (PFD) or pelvic floor dysfunction and slow transit constipation (STC + PFD) were significantly younger than the patients with irritable bowel syndrome (IBS) and tended to be younger than those with slow transit alone (STC). Over 80% of patients in each diagnostic category were women.

Symptoms. Spontaneous stools occurred least frequently in patients with STC, either alone or combined with PFD. Moreover these same patients had the least number of stools per week, whether spontaneous or facilitated by medication. Patients with slow transit as a component of their constipation therefore appeared to be more profoundly constipated than were patients who complained of constipation but who did not have slow transit.

There was a trend for more patients with a combined disorder (STC + PFD) to facilitate stooling using enemas compared with patients in the other groups. The type and frequency with which medications were used to aid stooling, however, did not appear to distinguish among the patient groups.

TABLE 1. *Characteristics of 277 Patients Presenting with Intractable Constipation by Diagnostic Category (Mean ± SD)*

Group	N	Age (yr)	Women (%)	Spontaneous Stools (% of patients)	Stools/wk (N)	Enemas (% of patients)	Laxatives (% of patients)	Facilitated Defecation (% of patients)
I Slow transit constipation	29	40 ± 16	84	3†	0.1 ± 0.2‡	44	67	11
II Pelvic floor dysfunction	37	36 ± 17*	89	18	1.2 ± 2.0	46	50	41§
III Slow transit constipation + pelvic floor dysfunction	14	33 ± 17*	89	0†	0.2 ± 0.4	78	67	11
IV Irritable bowel syndrome	197	43 ± 17	82	22	NA	50	60	17

* Groups II and III younger than Group IV ($p < 0.04$).

† Fewer patients in Groups I and III stooled spontaneously than patients in Group IV ($p < 0.02$).

‡ Stool frequency less than Group II ($p < 0.001$).

§ More patients in Group II facilitated defecation than patients in Groups I, III, and IV ($p < 0.02$).

Importantly patients with pure pelvic floor dysfunction (PFD) facilitated defecation digitally more often than did patients with STC alone or those with STC + PFD. These patients with PFD often reported straining endlessly on the toilet before digitally extracting the stool. Interestingly this was not a problem reported by patients with PFD and slow transit, perhaps because there was no stool in the rectum to extract.

Physiologic Results (Table 2)

Colon Transit. Patients with STC or STC + PFD had significantly slower colon transit times than did patients with pure PFD or IBS ($p < 0.05$).

Pelvic Floor and Anorectal Manometry. There were no differences among groups in mean resting or squeeze anal pressures or rectal capacity. The rectal-anal sphincter inhibitory response was present in all patients, thus ruling out Hirschsprung's disease in the entire group.

EMG. During defecation straining, some patients in all groups demonstrated paradoxical puborectal muscle contraction. In patients with PFD either alone or combined with STC (STC + PFD), 56% and 50% of patients had paradoxical contraction, respectively. In contrast only 43% of patients with STC alone and 34% of patients with irritable bowel syndrome had paradoxical activity in the puborectal muscle. It therefore appeared that abnormal motor activity of the puborectal muscle was not specific for PFD, although the highest incidence (56%) was in the group of patients with pure PFD.

Scintigraphic Balloon Topography. The magnitude of movements of the anorectal angle during defecation were similar across all groups. There was a trend for STC + PFD patients to open the anorectal angle less than all the other groups. The mean difference between rest and defecation angles in patients with STC + PFD was 3°, whereas it was 13° in patients with STC alone, 9° in patients with PFD alone, and 10° in IBS patients. None of these differences, however, were statistically significant.

Pelvic floor descent, however, was different among groups. Patients with PFD either alone or combined with STC had less descent of the pelvic floor than did patients with pure STC or IBS.

Scintigraphic Evacuation. Patients with STC + PFD had significantly less evacuation of radiolabeled artificial stool than did patients with STC or IBS.

Balloon Expulsion. Significantly fewer patients with PFD (19%) and patients with STC + PFD (13%) spontaneously evacuated the rectal balloon than did patients with either STC alone (60%) or irritable bowel syndrome (42%) ($p < 0.05$). Of the patients unable to evacuate spontaneously, patients with PFD or STC + PFD required greater weight to pass the intrarectal balloon than did patients with pure STC or IBS.

Defecating Proctograms. Eight patients, three with STC + PFD and five with STC, had symptoms of rectal fullness and tenesmus sufficient to warrant obtaining a defecating proctogram, even though previous studies, often including a defecating proctogram, had shown no diagnostic abnormalities. All three patients with STC + PFD were normal. Of the patients with STC alone, three patients were normal and one had a rectocele that did not significantly interfere with defecation. The remaining patient had a rectocele that impaired defecation; this patient underwent rectocele repair first and then abdominal colectomy and ileorectostomy.

Upper Gastrointestinal Manometry. Twenty-seven of two hundred seventy-seven patients had upper gastrointestinal manometry performed. Sixteen were normal and 11 showed evidence of small bowel pseudo-obstruction. Four of these patients underwent abdominal colectomy and ileorectostomy, including three patients from group I (STC) and one from group III (STC + PFD). Despite this diagnosis all four were doing well postoperatively, although one has required intermittent use of cisapride.

Management

Slow Transit Constipation. Having determined that all treatment modalities had been explored and had failed, and if the patient was psychologically fit, abdominal colectomy and re-anastomosis was performed in the STC

TABLE 2. *Results of Colonic and Pelvic Floor Function Tests in 277 Patients with Intractable Constipation (Mean ± SD)*

Group	Colon Transit (hr)	Pelvic Floor Descent (cm)	Scintigraphic Expulsion (% of instillate)	Balloon Expulsion (g needed to defecate)
I Slow transit constipation	117 ± 25*	2.4 ± 1.5	67 ± 21	70 ± 144
II Pelvic floor dysfunction	80 ± 38	1.4 ± 1.8†	58 ± 22	248 ± 182‖
III Slow transit constipation + pelvic floor dysfunction	118 ± 19*	0.6 ± 0.6‡	44 ± 23§	322 ± 203¶
IV Irritable bowel syndrome	68 ± 35	1.9 ± 1.6	66 ± 20	156 ± 187

* Transit slower than Groups II and IV ($p < 0.05$).
† Descent less than Group I ($p < 0.05$).
‡ Descent less than Groups I and IV ($p < 0.05$).
§ Percent expulsion less than Groups I and IV ($p < 0.05$).
‖ Weight required greater than Group I ($p < 0.05$).
¶ Weight required greater than Groups I and IV ($p < 0.05$).

group (n = 29). All patients underwent postoperative follow-up by a nurse and data clerk at 2 months, 6 months, and at yearly intervals thereafter.

Pelvic Floor Dysfunction. Patients with abnormal pelvic floor function (n = 37) underwent an intensive 10-day inpatient pelvic floor retraining program. Patients were admitted to an extended care facility of the hospital. Diet and activity were controlled. Pelvic floor retraining was performed after the manner of Bleijenberg and Kuijpers[25] and Weber et al.,[26] using biofeedback techniques. Patients were taught to relax the pelvic floor during straining and to correlate relaxation and pushing to achieve defecation.

Slow Transit Constipation + Pelvic Floor Dysfunction. Of the patients having a combined disorder (STC + PFD; n = 14), nine underwent pelvic floor retraining followed by ileorectostomy. Five patients completed retraining but have not had surgery performed.

Irritable Bowel Syndrome. Patients with normal studies were re-referred to their physicians with a diagnosis of IBS (n = 197) for further symptomatic care. No further diagnostic or any surgical maneuvers were performed in these patients.

Clinical Outcome

Thirty-six patients with STC alone and STC combined with PFD underwent colectomy and ileorectostomy; two underwent ileosigmoidostomy. The abdominal colon was removed using a conservative mesenteric dissection technique. The presacral space was entered, sparing the sympathetic nerves, the lateral rectal stalks loosened but not severed, and an ileorectal anastomosis into the proximal rectum was completed by a handsewn technique.

The colons from all patients underwent conventional histologic examination. There was no evidence of a myopathic process in any specimen; the smooth muscle appeared normal throughout. The mucosa harbored a mild to moderate degree of melanosis coli in only one patient. Special stains were performed in 25 specimens and ganglion cell and neuronal degenerative changes similar to those defined by Krishnamurthy et al.[27] were found in 15.

The mean (± SD) length of postoperative hospital stay was 12 ± 2 days for all patients. Bowel function returned in mean 6 ± 4 days after surgery. There were no deaths. The in-hospital morbidity rate (30-day) was low (Table 3). The complications that did occur were minor. Five patients had a prolonged ileus; such patients usually had bowel function return early, but, because the abdomen remained distended, they required prolonged nasogastric decompression. Hospital stay in this group of ileus patients, however, was no longer than that of patients without an ileus. No patient required reoperation during the immediate postoperative period.

One of the two patients who had had an ileosigmoidostomy developed recurrent constipation within 4 months of the original operation. This patient was reoperated on and an ileorectostomy was performed.

TABLE 3. *Early and Late Complications of Abdominal Colectomy and Ileorectostomy in Patients Operated on for Slow Transit (Group I) or Slow Transit + Pelvic Floor Dysfunction (Group III)*

Complication	No. of Patients (%)
Early	
Prolonged ileus	5 (13)
Wound infection	1 (3)
Urinary tract infection	2 (5)
Reoperation	0
Incontinence	0
Late	
Small bowel obstruction (no. episodes/no. patients/ patients reoperated)	6/4/3
Ventral hernia	1 (3)
Pancreatitis (resolved)	1 (3)
Incontinence	0

The most frequent long-term complication was small bowel obstruction. Six episodes of small bowel obstruction occurred in four different patients from 2 months to 4 years after operation. Three of these four patients required reoperation to lyse adhesions.

Follow-up was complete in all but one patient. The mean length of follow-up was 20 months. Except for the patient converted from ileosigmoidostomy, no patient had recurrent constipation. One patient with documented small intestinal pseudo-obstruction did, however, use laxatives for a few weeks postoperatively. The one patient who had an ileorectostomy after failed ileosigmoidostomy has remained free of constipation.

The postoperative stool frequency among all patients declined from 4 per 24 hours to 2 per 24 hours by 3 years (Fig. 2). Approximately two thirds of patients passed solid stools soon after operation, rising to 100% of patients at the 3-year follow-up (Fig. 3). Gratifyingly the number of patients with liquid stools even at the 2-month interval was less than 10%. The requirement for bulking agents declined from 40% at 2 months to none by 3 years after operation (Fig. 4). Importantly Lomotil® or Imodium® were used by only a few patients early after their operation and no patient used them after 1 year (Fig. 4). At every interval of follow-up from 2 months to 3 years, all patients were continent.

Discussion

We found that tests of colonic and pelvic floor function, administered prospectively in patients with intractable constipation, delineated patients with slow transit constipation alone or patients with slow transit constipation and pelvic floor dysfunction, who were amenable to operative management, from those with pelvic floor dys-

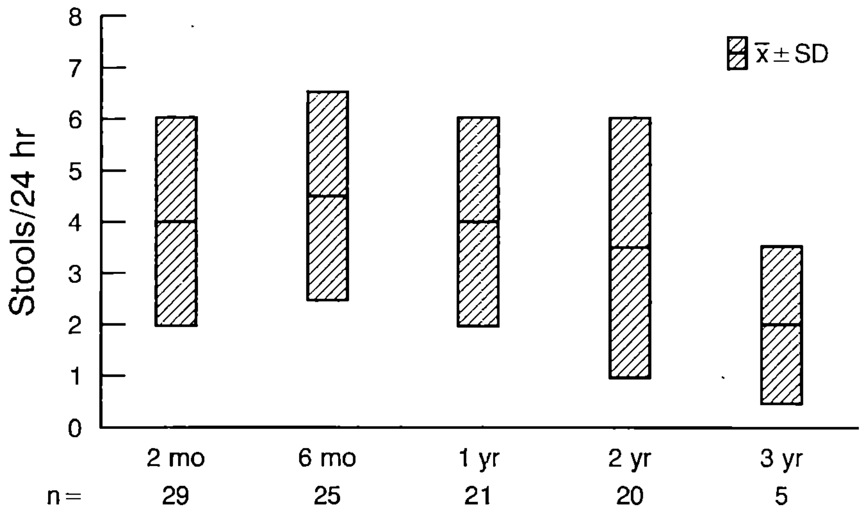

FIG. 2. Plot of stool frequency of 38 patients from 2 months to 3 years after abdominal colectomy and ileorectostomy for slow transit constipation (Group I; n = 29) and slow transit + pelvic floor dysfunction (Group III; n = 9).

function alone and irritable bowel syndrome, who were not. These observations confirm and extend those of others who have demonstrated several different causes for chronic constipation in adults.[3-7]

Hinton and colleagues[28] described the abnormally slow movement of radiopaque markers through the colon and later labeled such patients as having slow transit constipation.[3] The cause of slow transit constipation is unknown, but may be related to abnormalities of smooth muscle innervation.[27] Patients with profoundly slow transit as the sole cause of their constipation are likely to benefit from colectomy.[29]

Conversely Preston and Lennard-Jones,[4] Read et al.,[5] Turnbull and colleagues[6] and Barnes et al.,[30] in a series of interesting observations, have shown that impaired defecation is another significant functional cause of constipation. The typical patient is a young woman with severe chronic intractable constipation who describes difficulty with expulsion of stool, failure to pass an artificial stool, or failure to pass barium or a rectal balloon. The cause of impaired defecation was hypothesized to be failure of the puborectal muscle and external anal sphincter to relax on straining.[4] This was termed "anismus." Patients who underwent surgery for anismus, which entailed division of the puborectalis muscle, fared poorly.[30,31]

In addition to anismus, defecation disturbances are caused by anatomic abnormalities, such as Hirschsprung's disease, occult and complete rectal prolapse, descending perineum syndrome, and functionally significant rectoceles. Among these surgery for Hirschsprung's disease, complete rectal prolapse, and rectocele is likely to be successful.

Finally irritable bowel syndrome (IBS), a poorly understood problem, has been implicated as a key player in causing constipation in large numbers of patients.[32] These patients have constipation alone (constipation-predomi-

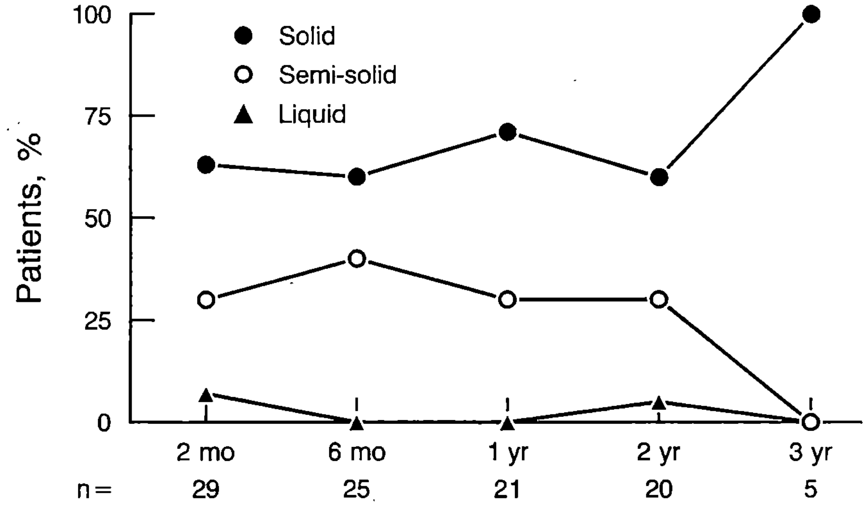

FIG. 3. Plot of stool consistency among 38 patients from 2 months to 3 years after abdominal colectomy and ileorectostomy for slow transit constipation (Group I; n = 29) and slow transit + pelvic floor dysfunction (Group III; n = 9).

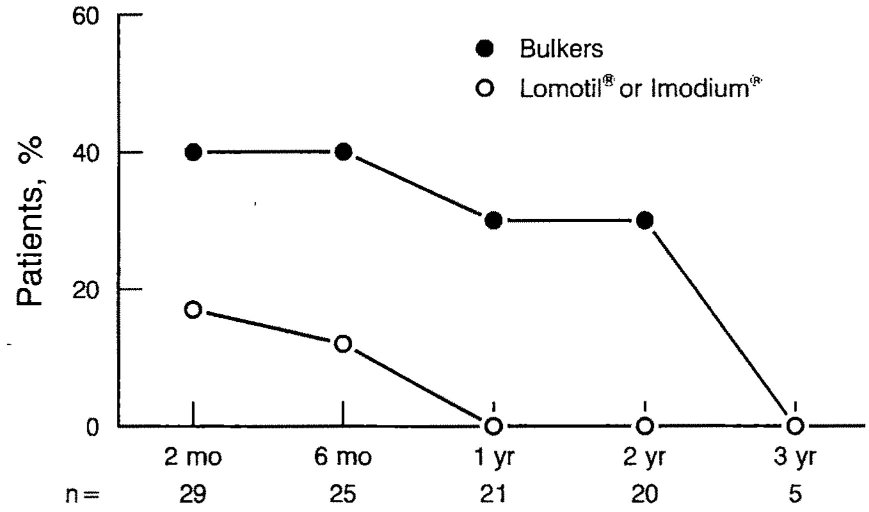

FIG. 4. Use of bulking agents and the hypomotility agents Lomotil (diphenoxylate) and/or Imodium (loperamide) among 38 patients from 2 months to 3 years after abdominal colectomy and ileorectostomy for slow transit constipation (Group I; n = 29) and slow transit + pelvic floor dysfunction (Group III; n = 9).

nant IBS) or alternating constipation and diarrhea. Characteristically patients complain excessively of abdominal pain, and pass small hard pellets of stool but usually have normal colon transit.[33] Our findings in patients with IBS, the largest group of patients evaluated by far, agree with these observations. Patients with IBS respond poorly to surgery; indeed ileorectostomy may be a spectacular failure, with end ileostomy the final result. We therefore did not operate on any of these patients.

There is little doubt that the key to selecting patients who will benefit from surgery is objective physiologic testing. To delineate causes we measured colonic and pelvic floor function quantitatively using tests previously validated in pilot studies in our laboratory. We found that these tests appeared to delineate patients into causative and treatment groups in a relatively straightforward and reliable manner, with predictable results. Importantly selection of patients who were candidates for operation and who subsequently did well was facilitated.

Certainly Keighley[8] is right in warning surgeons to be conservative when intervening surgically in patients with constipation. This stance and our conservative philosophy was based on the somewhat checkered history of surgery for constipation. Indeed results of surgery for constipation vary dramatically even in the most recent literature.[10,12,34-36] One major reason for this variability is that mechanisms of constipation were rarely investigated before operation; a quite heterogeneous patient population therefore was operated on, with highly variable outcomes the inevitable result. One small study did divide patients into slow transit and "outlet obstruction" groups.[29] Patients with slow transit did well after ileorectostomy, as they did in our series.

Even though the patients studied were already highly selected, systematic evaluation identified a quantifiable abnormality of colonic or pelvic floor function or both in only 83 of 277 patients (30%). The rest had normal studies. Twenty-nine (10%) of the entire group of 277 patients had slow transit constipation, 37 (13%) had pelvic floor dysfunction, and 14 (5%) had both; therefore only 15% of all patients presenting with intractable severe constipation were deemed possible candidates for surgery. It is not surprising therefore that the results of previous surgical series, which did not evaluate patients prospectively and thus did not categorize them preoperatively, were so variable.

When performed in patients with slow transit and normal pelvic floor function, abdominal colectomy and ileorectostomy was safe and effective; patients had four stools per day from 2 months to 2 years, decreasing to 2 per day at 3 years. No patient was incontinent. Importantly constipation has not recurred in this group. These results are similar to those reported by several authors,[29,37,38] but stand in direct contrast to others.[12,39,40] Although the mean follow-up was 20 months in our patients, function could indeed deteriorate over time and constipation could recur; continuing evaluation is therefore mandatory.

Among the two patients who underwent ileosigmoidostomy instead of ileorectostomy, one failed and an ileorectostomy had to be constructed. These observations agree with those of Vasilevsky et al.[34] and Belliveau et al.,[41] who reported relatively poor results in patients undergoing ileosigmoidostomy. Indeed in our studies segmental colon transits did not show a predominant degree of slowing in any one segment in particular; less than total colectomy with anastomosis into the rectum proper results in continuing constipation in most patients and should not be performed.

Colectomy has not been advocated in patients with small intestinal pseudo-obstruction because symptoms of constipation usually recur.[24] The four patients with manometrically documented small bowel pseudo-obstruction operated on in this series, however, did well; one took a laxative for 3 weeks and stopped and one has been given cisapride intermittently for unknown reasons by her referring physician despite a lack of complaints. There is little doubt that problems might occur in these patients in the future, but at a mean of 20 months after operation, bowel function is stable and predictable.

Fourteen patients in our study had "combined" slow transit constipation and pelvic floor dysfunction. It seems possible that these patients may have failed ileorectostomy alone, because defecation abnormalities would have persisted postoperatively. Indeed continuing constipation has been reported to occur after ileorectal anastomosis for constipation[2,39,40]; patients who are constipated after ileorectostomy likely have abnormal pelvic floor function. Our patients therefore underwent pelvic floor retraining first. Thirteen of fourteen (93%) learned to stool spontaneously during this program and thus became candidates for colectomy. The results of ileorectostomy in the nine patients operated on did not differ from that of the larger group of pure slow transit patients (STC). No patient had recurrent constipation or incontinence.

Frequent watery stools and incontinence have been reported after ileorectostomy for constipation.[42] This did not occur in our series. One explanation might be that we did not, to the best of our knowledge, operate on patients with IBS.

In summary tests of colonic and pelvic floor function distinguished intractably constipated patients with slow colonic transit who were candidates for operation from those with pelvic floor dysfunction or irritable bowel syndrome who were not. Patients with pelvic floor dysfunction underwent pelvic floor retraining. Results in this group of patients await further analysis. A subgroup of patients with PFD and SCT, however, underwent pelvic floor retraining successfully first and then operation. Patients with IBS continued with medical management.

We concluded that abdominal colectomy and ileorectostomy in patients shown to have slow transit alone or combined pelvic floor dysfunction and slow transit constipation was safe and effective and resulted in prompt and prolonged relief of constipation.

References

1. Drossman DA, Sandler RS, McKee DC, Lovitz AJ. Bowel patterns among patients not seeking health care. Gastroenterology 1982; 83:529–534.
2. Pemberton JH, Levin KE. The surgery of intractable constipation. Current Practice in Surgery 1990; 2:117–124.
3. Preston DM, Lennard-Jones JE. Severe constipation of young women: Idiopathic slow transit constipation. Gut 1986; 27:41–48.
4. Preston DM, Lennard-Jones JE. Anismus in chronic constipation. Dig Dis Sci 1985; 30:413–418.
5. Read NW, Timms JM, Barfield LJ, et al. Impairment of defecation in young women with severe constipation. Gastroenterology 1986; 90:53–60.
6. Turnbull GK, Lennard-Jones JE, Bartram CI. Failure of rectal expulsion as a cause of constipation: Why fibre and laxatives sometimes fail. Lancet 1986 i:767–769.
7. Martelli H, Devroede G, Arhan P, Duquay C. Mechanisms of idiopathic constipation: Outlet obstruction. Gastroenterology 1978; 75:623–631.
8. Keighley MRB. Surgery for constipation. Br J Surg 1988; 75:625–626.
9. Kamm MA, Hawley PR, Lennard-Jones JE. Is colectomy for severe idiopathic constipation a success? Gut 1987; 28:A1343.
10. Kamm MA. The surgical treatment of severe idiopathic constipation. Int J Colorectal Dis 1987; 2:229–235.
11. Leon SH, Krishnamurthy S, Schuffler MD. Subtotal colectomy for severe idiopathic constipation. A follow-up study of 13 patients. Dig Dis Sci 1987; 32:1249–1254.
12. Yoshioka K, Keighley MRB. Clinical results of colectomy for severe constipation. Br J Surg 1989; 76:600–604.
13. Metcalf AM, Phillips SF, Zinsmeister AR, et al. Simplified assessment of segmental colonic transit. Gastroenterology 1987; 92:40–47.
14. Taylor BM, Beart RW Jr, Phillips SF. Longitudinal and radial variation of pressure in the human anal sphincter. Gastroenterology 1984; 86:693–697.
15. Yoshioka K, Keighley MRB. Randomized trial comparing anorectal myectomy and controlled anal dilatation for outlet obstruction. Br J Surg 1987; 74:1125–1129.
16. Rutler KRP. Electromyographic changes in certain pelvic floor abnormalities. Proc R Soc Med 1974; 67:53–56.
17. Jones PW, Lubowski DZ, Swash M, Henry MM. Is paradoxical contraction of the puborectalis muscle of functional importance? Dis Colon Rectum 1987; 30:667–670.
18. Barkel DC, Pemberton JH, Pezim ME, et al. Scintigraphic assessment of the anorectal angle in health and after ileal pouch-anal anastomosis. Ann Surg 1988; 208:42–49.
19. Pezim ME, Pemberton JH, Phillips SF. The immobile perineum: Pathophysiologic implications in severe constipation. Dig Dis Sci 1987: 32:924.
20. O'Connell PR, Kelly KA, Brown M. Scintigraphic assessment of neorectal motor function. J Nucl Med 1986; 27:460–464.
21. Preston DM, Lennard-Jones JE, Thomas BM. The balloon proctogram. Br J Surg 1984; 71:29–32.
22. Mahieu P, Pringot J, Bodart P. Defecography. I and II. Description of a new procedure and results in normal patients: and contribution to the diagnosis of defecation disorders. Gastrointest Radiol 1984; 9:247–261.
23. Shorvan PJ, McHugh F, Sommers S, Stevenson GW. Defecographic findings in young healthy volunteers. Gut 1987; 29:A1361–A1362.
24. Stanghellini V, Camilleri M, Malagelada J-R. Chronic idiopathic intestinal pseudo-obstruction: clinical and intestinal manometric findings. Gut 1987; 28:5–12.
25. Bleijenberg G, Kuijpers HC. Treatment of the spastic pelvic floor syndrome with biofeedback. Dis Colon Rectum 1987; 30:108–111.
26. Weber J, Dugnotte PH, Touchais JY, et al. Biofeedback training for constipation in adults and children. Dis Colon Rectum 1987; 30: 844–846.
27. Krishnamurthy S, Schuffler MD, Rohrmann CA, Pope CE. Severe idiopathic constipation is associated with a distinctive abnormality of the colonic myenteric plexus. Gastroenterology 1985; 88:26–34.
28. Hinton JM, Lennard-Jones JE, Young AC. A new method for studying gut transit times using radio-opaque markers. Gut 1969; 10:842–847.
29. Keighley MRB, Shouler P. Outlet syndrome: Is there a surgical option. J R Soc Med 1984; 77:559–563.
30. Barnes PRH, Hawley PR, Preston DM, Lennard-Jones JE. Experience of posterior division of the puborectalis muscle in the management of chronic constipation. Br J Surg 1985; 72:475–477.
31. Kamm MA, Hawley PR, Lennard-Jones JE. Lateral puborectalis

division in the management of severe constipation. Br J Surg 1988; 75:661–663.
32. Read NW, Timms JM. Defecation and the pathophysiology of constipation. Clin Gastroenterol 1986; 15:937–965.
33. Lanfranchi GA, Bazzocchi G, Brignola C, et al. Different patterns of intestinal transit time and anorectal motility in painful and painless chronic constipation. Gut 1984; 25:1352–1357.
34. Vasilevsky CA, Remer FD, Balcos EG, et al. Is subtotal colectomy a viable option in the management of chronic constipation. Dis Colon Rectum 1988; 31:679–681.
35. Klatt GR. Role of subtotal colectomy in the treatment of incapacitating constipation. Am J Surg 1983; 145:623–625.
36. Walsh PV, Peebles-Brown G, Watkinson G. Colectomy for slow transit constipation. Ann R Coll Surg 1987; 69:71–75.
37. Beck DE, Jagelman DG, Fazio VW. The surgery of idiopathic constipation. Gastroenterol Clin North Am 1987; 16:143–156.
38. Hughes ESR, McDermott FT, Johnson WR, Polglase AL. Surgery for constipation. Aust N Z J Surg 1981; 51:144–148.
39. Kamm MM, Hawley PR, Lennard-Jones JE. Is colectomy for severe idiopathic constipation a success? Gut 1987; 28:A1343.
40. Preston DM, Hawley PR, Lennard-Jones JE, Todd IP. Results of colectomy for severe idiopathic constipation in women (Arbuthnot Lanes' disease). Br J Surg 1984; 71:547–552.
41. Belliveau P, Goldberg SM, Rothenberg D, Nivatvongs S. Idiopathic acquired megacolon: The value of subtotal colectomy. Dis Colon Rectum 1982; 55:118–121.
42. Kamm MA, Hawley PR, Lennard-Jones JE. Outcome of colectomy for severe idiopathic constipation. Gut 1988; 29:969–973.

DISCUSSION

DR. HARVEY J. SUGERMAN (Richmond, Virginia): I believe this is an important study by Dr. Pemberton that documents a small subset of patients with severe intractable constipation who have slow colonic transit and will benefit from a total abdominal colectomy and ileoproctostomy. Other studies have shown clearly that partial colectomy, as in one of their patients, is destined to fail, and routine studies, such as barium enema and colonoscopy, although necessary to rule out other causes of constipation, are normal in those with slow transit constipation.

The prime diagnostic test is rather simple; namely the passage of little pieces of cut-up radiopaque nasogastric tubing into the rectum within a reasonable period. The other critical test is to detect a rectal-anal sphincter inhibitory response to balloon distension using anal manometry to rule out Hirschsprung's.

I have several questions regarding the evaluation and management of these patients. Which of the other tests you performed—defecography, scintillation topography, balloon evacuation, scintigraphic evacuation, EMG of the puborectalis muscle, etc.—are necessary in the evaluation of these patients?

Although similar results were noted by Dr. Becker's group in a much smaller number of patients (Zenilman ME, Dunnegan DL, Soper NJ, Becker JM. Successful surgical treatment of idiopathic colonic dysmotility. Arch Surg 1989; 124:947–951) presented to the Western Surgical Association 2 years ago, Yoshioka and Keighley (Yoshioka K, Heighley MRB. Clinical results of colectomy for severe constipation. Br J Surgery 1989; 76:600–604) noted that 12 of 40 of their patients so treated required additional surgery, a permanent ileostomy in 6 of the 40, due to severe diarrhea, and a completion proctectomy and ileal pouch anal anastomosis in another six because of the recurrence of severe constipation. Mr. Nichols (Nichols RJ, Kamm MA. Proctocolectomy with restorative ileoanal reservoir for severe idiopathic constipation. Report of two cases. Dis Colon Rectum 1988; 31:968–969) also noted recurrence of severe constipation in two patients who had a subsequent excellent functional result with the ileo-anal procedure.

Do you believe the high incidence of diarrhea after colectomy and the ileo-proctostomy noted by Yoshioka and Keighley was the result of anal myomectomy performed in a number of their patients? Do you believe that there is any place for anal myomectomy in patients with pelvic floor dysfunction?

As your longest follow-up is only 4 years with a mean of 20 months, do you believe that is enough time to be certain that recurrent constipation will not occur? Finally do you believe there is any place for colectomy, proctectomy, and the ileo-anal procedure for severe constipation?

I very much enjoyed reading this paper and feel that it will provide a significant aid in the evaluation and management of this constipation problem.

DR. MARTIN S. LITWIN (New Orleans, Louisiana): This paper is unusual, because it calls to our attention a problem that surrounds us almost every day and that we frequently refuse or fail to recognize.

Intermittent abdominal distension with constipation and vague abdominal pain relieved by passage of flatus and stool are common chronic complaints, particularly among middle-aged women. Their symptoms are vague and their responses to conservative treatment, including laxatives and stool softeners, are so poor that they are often diagnosed as having intestinal problems of psychosomatic origin. Irritable bowel syndrome is an example.

Frequently they have earlier undergone hysterectomy or other pelvic procedures. In 1984 we reported four such patients, all of whom had earlier undergone gynecologic operations (*Am Surg* 1984; 50:479–481). In our patients their complaints were based on the presence of pelvic adhesions resulting from their earlier gynecologic operations. These adhesions were at the internal operative sites that had not been reperitonealized and severely distorted their sigmoid colon. This in turn produced partial and chronic sigmoid colon obstruction.

All the diagnoses were made during colonoscopy. At that time we noted sharp angulation of the sigmoid that was caused by contracture of the adhesions at the sites that had not been reperitonealized. At the time of colonoscopy, all patients noted induction of typical crampy pain not relieved by intravenous glucagon when the angulation was either passed or partially straightened with the colonoscope.

Once the diagnoses were made, treatment of these patients consisted of lysis of adhesions, restoration of the colon to its normal anatomic position, and reperitonealization of the exposed pelvic surfaces.

I would be interested to know from Dr. Pemberton the history of previous pelvic surgery, the success rate of the 183 patients that had the irritable bowel syndrome, and the method by which pelvic floor retraining was accomplished.

Additionally I would appreciate information as to his definition of constipation. Patients in his study appeared to me to have been relatively young.

DR. STANLEY M. GOLDBERG (Minneapolis, Minnesota): This is a most diligent attempt to develop a coherent physiologic directed treatment approach to the constipated patient.

We completely support the comprehensive evaluation of the constipated patient. We also have difficulty in classifying this condition, which has a recognizable overlap of physiologic abnormalities.

This brings me to my first question. In your group of patients with slow transit time constipation, at least 40% had an abnormality of their pelvic floor function, either an inappropriate puborectalis in 43%, or 40% failed the balloon expulsion test. Is it possible that a proportion of these patients with pelvic floor dysfunction would have benefited from biofeedback alone and avoided a colectomy?

Our experience has also led us to be very cautious in recommending subtotal colectomy for patients with pelvic floor dysfunction. We recently did anal-rectal physiologic assessments on 18 patients before subtotal colectomy. Our postoperative complication rate is similar. We had an 11% small bowel obstruction rate requiring laparotomy. Three patients with nonrelaxing puborectalis muscle underwent subtotal colectomy, and these three had a suboptimal result. We defined a suboptimal result as a subsequent need for an ileostomy or, quote, excessive straining at stool.

This brings me to my final question. Han Kuijpers of Nijmegen has shown that 14 out of 16 patients with pelvic floor dysfunction and slow transit time constipation were successfully managed with biofeedback therapy alone. I would be interested to know why all nine patients in your study, with a combined slow transit time type of constipation and pelvic floor dysfunction, required a subtotal colectomy?

DR. ROBERT E. CONDON (Milwaukee, Wisconsin): As all of you are aware, for years patients with chronic constipation syndromes have had to put up with the frustrations of ineffective treatment, and their physicians have been equally frustrated by inability to have any real impact on the management of these disorders.

Further patients have been subjected to the denigration associated with the simplistic and now largely discredited Freudian hypothesis that the problem was due to faulty toilet training in childhood, leading to a defect in the adult personality. In truth it is probably the other way around. As in every chronic disease, over the years the persisting symptoms tend to have a disintegrating impact on the persona of the victim.

So defining the true cause of chronic disease and especially of this particular syndrome is important, and the answers lie increasingly, it is obvious, not in psychotherapy but in physiology.

Mr. President, the reason for the interest among many of us in these disorders is exemplified by the data that have been presented today. Among 277 patients, small subsets with treatable disorders were identified, but 197 of those 277 patients were sent home without relief. Obviously a substantial problem remains unsolved.

Now some observations and some questions for Dr. Pemberton. Some of my questions have already been posed, and I will try not to repeat them. But one of them has to do, John, with the major sex bias in your data. Over 80% of your patients are women. The question about previous pelvic surgery has already been posed. Do you have information about the obstetric history and whether or not obstetrical birth injury played a role in the genesis of pelvic floor dysfunction?

Second the diagnosis of pelvic floor dysfunction was established using a large battery of invasive tests, six in all. While I assume that not all tests were applied to all patients, the approach does seem complex and perhaps redundant. So what were the final minimal criteria for the establishment of the diagnosis of pelvic floor dysfunction? And based on your experience, can you recommend a less-complex diagnostic workup to us?

Treatment of pelvic floor dysfunction using biofeedback seems to have been a great success in your experience, but that is not true of all experiences. Can you give us some further details, particularly of the duration of the training program?

Finally you observed in 15 of 25 colons resected for slow transit—this information is in the manuscript and was not presented this morning—abnormal ganglion cells and neuronal degeneration as previously reported by Pope as associated with chronic constipation. John, do you think these abnormalities in bowel innervation are secondary effects, or are they primary and perhaps causative in the syndrome of slow transit constipation?

DR. W. HARDY HENDREN, III (Boston, Massachusetts): Dr. Pemberton has shown us some very useful tests in evaluating the patient with constipation in not only his presentation but in his manuscript, which he allowed me to read.

I would like to say that constipation might seen to be a mundane problem, but it is a terrible problem in the age group in which I work, the child and the young adolescent, leading to great morbidity and in failure to thrive for many children, as well as tremendous psychological problems.

I would like to stress one parameter in constipation that has not been mentioned, and that is to do a very careful physical examination of the patient's bottom, because there are many constipated patients of all ages, in my experience, in whom there is slight anterior displacement of the anus.

If your anus is a bit further forward than it should be, then there is a shelf that on rectal examination does not feel abnormal, on barium examination does not look abnormal, even in the lateral view frequently, but that functionally causes obstruction that can be relieved by a simple posterior anoplasty, which we routinely perform on an outpatient basis.

I reported a series of 134 of those in 1978, and in the series there was a 42-year-old woman who came into the hospital with perforation of her colon from chronic constipation, and this entity that she had lived with, causing constipation all of her life, had been totally missed, including four occasions in which she had been in stirrups for vaginal delivery of four children, and her anomaly had never been noted. It was not necessary to do anything more for her than a posterior anoplasty.

Further we have lately recognized that among children with imperforate anus who have had successful surgery, there remains dilatation of the sigmoid colon in some, and this causes constipation. The problem is that the dilated bowel cannot contract and it cannot propel the bolus of feces properly, and patients are constipated. We have found that resecting those sigmoids, just as Dr. Pemberton has shown us for his group of patients, frequently cures this constipation problem.

I would like to ask you three questions. First, does your series of constipated patients at the Mayo Clinic also include some patients who have some degree of subtle or obvious anal ectopia?

Second I notice that your patients seem to cluster the 30 to 40 age group, and the oldest was a late teenager. Is that because you have patient selection by age at the Mayo Clinic and therefore are not seeing the kids, or do you think this is an acquired problem?

And thirdly, as Dr. Condon mentioned, you have a preponderance of females, from 80% to 89%. Do you have an explanation for that? Because our mix in the cases that we see is about 50/50 males *versus* females in the pediatric and adolescent age group.

Finally I would like to thank you for this presentation, and to thank you for pointing out an area in which the general surgeon can still work, which I think is important in this age in which everybody is encroaching on and narrowing what we can do to a very small spectrum.

DR. JOHN H. PEMBERTON (Closing discussion): Thank you for all the excellent questions. I will try to group them and answer them as expeditiously as possible.

Concerning the number of tests, the reason we performed multiple studies was that we did not know which tests would be the most specific for pelvic floor abnormalities; thus the battery of studies. If you press me to tell you which of the tests I would really want performed, which several of you just did, I would want the colon transit time for sure. Next I would want to know the degree of the pelvic floor descent on straining, and I would want to know whether or not they could pass a balloon either spontaneously or with less than 200 g of weight. These are the tests of central importance. Anorectal manometry of course, has a central role to play as a screening test to rule out Hirschsprung's disease and to determine rectal vault compliance. A diagnosis of pelvic floor dysfunction, however, cannot be made on the basis of anorectal manometry alone.

Dr. Sugarman asked about ileo-anal anastomosis for constipation. This seems to be a very aggressive approach. In the literature the patients in whom ileo-anal anastomosis has been performed had either megabowel or an unsuccessful previous operation. In these patients, particularly those with megabowel, such an aggressive approach is probably warranted. It is important to remember that although some of these ileo-anal patients have done well, others continued to have symptoms such as abdominal pain even though all of the large bowel and rectum have been removed.

Dr. Sugarman, we are hopeful that the rate of recurrent constipation stays nil; if not, then an operation designed to remove the rectum, yet maintain fecal incontinence, such as ileo-anal anastomosis, may be required.

Is there a reasonable operation for pelvic floor dysfunction? I do not think so. Internal anal sphincter myectomy and division of the puborectalis muscle have been studied and were proposed as beneficial operations. The results, however, have been very unpredictable and sometimes frankly not good at all.

Dr. Litwin brought up an interesting group of patients, those who have undergone gynecologic surgery. The particular patient that he described, it is hoped, would have been diagnosed during the conventional workup, by colonoscopy or barium enema, and therefore would have fallen into the "structural" abnormality group and not the "functional" abnormality group. Dr. Litwin also asked about our definition of constipation. The best general definition is the following: less than two stools per week or straining more than 25% of the time when having a bowel movement.

Dr. Condon asked about pelvic floor retraining. Our program is an inpatient 10-day training session that combines several different management approaches. One is biofeedback (which consist of sensation retraining, relaxation exercises, and coordination retraining) performed by the Physical Medicine and Rehabilitation Department. Psychological testing is performed by a single psychologist, and dietitians observe and manage intake precisely. This multimodality approach has been well received by the patients, and results are predictably good in the two groups of patients retrained. In a small group of patients with descending perineum syndrome, not included in this series, the results of pelvic floor retraining have been far less predictable, but nonetheless, improvement has occurred in more than one half of the patients.

Dr. Goldberg, you are right; some of the patients with slow transit constipation alone did have evidence of abnormal pelvic floor dysfunction on one or two of the pelvic floor studies. That is precisely why we performed so many studies. During the pelvic floor testing, if only one of these tests was abnormal, we tended to discount it. The more studies we had that were positive, the more likely the patients would fall into the pelvic floor dysfunction group. Once again no *one* study was pathognomonic of pelvic floor dysfunction. The patients in the combined group, after undergoing pelvic floor retraining, continued to have symptoms of slow transit constipation confirmed by repeat marker studies. Thus in this group of patients, biofeedback alone, although normalizing pelvic floor function, did not alleviate the constipation.

Dr. Condon, I do not know if patients with constipation have a problem of innervation of the colon primarily, but certainly the evidence is accumulating that this might indeed be the cause. In our series 15 of 25 colon specimens submitted for special studies showed ganglion cell and axonal degeneration.

Dr. Hendron asked why there was so many women in our series. Women constitute the preponderance of patients in every series reported to date. I have no idea why this is the case. In children the sex ratio indeed is 1:1. Constipation patients over 18 years of age, however, are nearly all women. At Mayo our group only evaluates and treats patients over 18 years of age.

Dr. Hendron also offered a fascinating observation, that of an anteriorly displaced anus. I admit that I did not look for this abnormality in our patients and therefore the incidence among adult patients at Mayo remains unknown. Indeed we will look for this in the future.

The reason that we did not have a high incidence of diarrhea in our patients after ileorectostomy was, that to the best of our knowledge, we did not operate on patients with irritable bowel syndrome. Patients who have irritable bowel syndrome and who undergo aggressive surgical intervention for constipation will have poor results. That is why we are careful to compulsively rule out irritable bowel syndrome in our patients.

Again thank you very much for all of the comments and thank the association for allowing me to present these data.

Duodenal Ulcer

Discovery of a New Mechanism and Development of Angiogenic Therapy That Accelerates Healing

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The complete purification of the first angiogenic molecule, basic fibroblast growth factor (bFGF), was carried out in the authors' laboratory in 1983. Application of this peptide to chronic wounds enhances angiogenesis and accelerates wound healing. The authors showed that an acid-stable form of bFGF (*i.e.*, bFGF-CS23) could be administered orally to rats with duodenal ulcers. The peptide promoted a ninefold increase of angiogenesis in the ulcer bed and accelerated ulcer healing more potently than cimetidine. Basic fibroblast growth factor did not reduce gastric acid. The authors now show that bFGF exists as a naturally occurring peptide in rat and human gastric and duodenal mucosa. This endogenous bFGF is present also in the bed of chronic ulcers in rats. Sucralfate binds bFGF and protects it from acid degradation. The sucralfate is angiogenic, based on its affinity for bFGF. When sucralfate is administered orally to rats, it significantly elevates the level of bFGF in the ulcer bed. Cimetidine, by its capacity to reduce gastric acid, also elevates bFGF in the ulcer bed. A hypothetical model is proposed in which prevention of ulcer formation or accelerated healing of ulcers by conventional therapies may be FGF dependent. Acid-stable bFGF-CS23 may be considered as a form of replacement therapy in the treatment of duodenal ulcers.

THE FIELD OF angiogenesis research, which has been pursued in the laboratory during the past 20 years,[1-5] is beginning to yield clinical applications. In the area of cancer diagnosis, for example, the risk of distant metastasis in breast cancer now can be predicted by measurement of angiogenesis in biopsy specimens.[6] The first clinical use of an angiogenesis inhibitor has induced regression of life-threatening hemangiomas in children,[7,8] and a novel angiogenesis inhibitor is undergoing preclinical testing for use against solid tumors that have failed conventional therapy.[9] A clinical trial of "therapeutic angiogenesis" was recently reported in which healing was accelerated in chronic wounds by topical application of an angiogenic peptide.[10]

In this report we describe the development of another modality of therapeutic angiogenesis: the accelerated healing of experimental duodenal ulcers by an orally administered angiogenic growth factor.

Considerable evidence indicates that angiogenesis plays an important role in wound healing.[11-13] When neovascularization is suppressed or delayed, wound healing itself is retarded.[14,15] Recent experimental studies suggest that topical application of an angiogenic peptide such as basic fibroblast growth factor (bFGF) can promote the formation of vascularized granulation tissue.[16-19] Furthermore healing of experimental wounds can be accelerated by topical application of bFGF.[20-24]

Rationale for the Studies Reported Here

Chronic duodenal ulcers share many similarities with chronic wounds. Both lack an epithelial covering and contain inflammatory cells, exposed collagen, necrotic debris, and granulation tissue. Granulation tissue is composed mainly of capillary blood vessels, monocytes, and fibroblasts. It is essential for the healing of chronic ulcers. Therefore we asked, "Can the healing of chronic duodenal ulcers be accelerated by stimulating angiogenesis in the ulcer bed?"

To test this hypothesis we administered bFGF orally to rats with chronic duodenal ulcers to achieve topical application of the peptide to the ulcer bed. Basic fibroblast growth factor is one of the most potent of seven angiogenic

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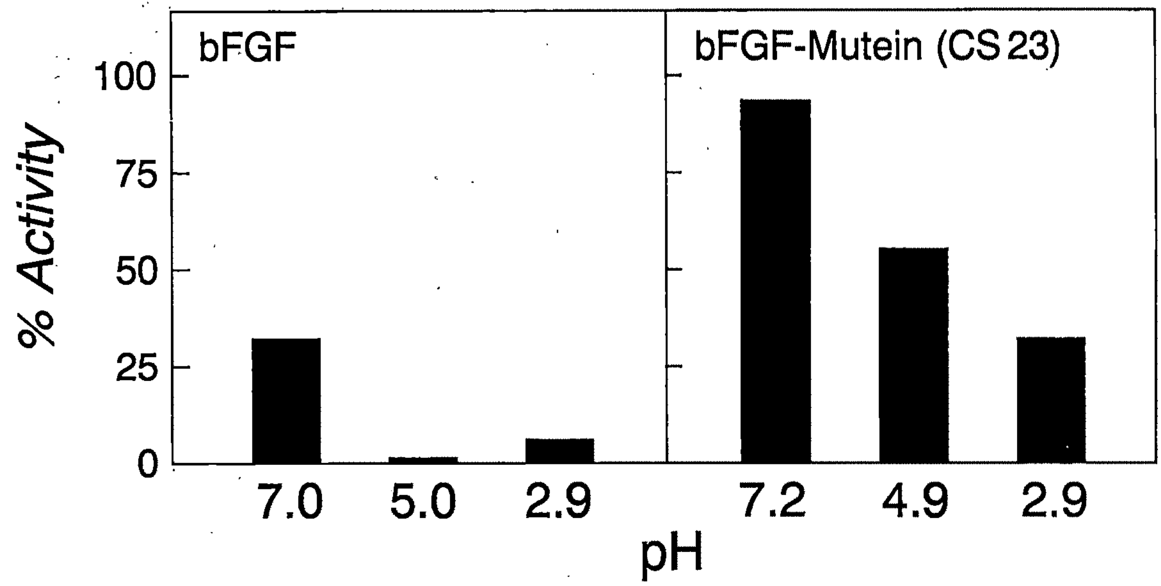


FIG. 1. Degradation of bFGF by HCl at 2 hours. Recombinant human bFGF (= wild-type bFGF [bFGF-w]), was incubated at various pHs for 2 hours and its mitogenic activity on endothelial cells was measured. The bFGF CS23 mutein was incubated for the same time at similar pH levels, and its mitogenic activity was measured in the same manner on endothelial cells. The bFGF CS23 mutein is significantly more acid stable than the wild-type bFGF. Redrawn from Seno *et al.*[27]

molecules that have been completely sequenced and cloned.[3] It has a high affinity for heparin, and we originally purified it on the basis of its heparin-binding capacity.[25] Basic fibroblast growth factor is a direct mitogen for vascular endothelial cells as well as for fibroblasts, smooth muscle cells, and certain epithelial cells.[26] Because biologic activity of bFGF is rapidly degraded at pH 7.0 or below[27] (Fig. 1), we employed an acid-stable form of bFGF, produced by site-specific mutagenesis in which the second and third of the four cysteines within the peptide were substituted by serines (bFGF-CS23 mutein)[27] (Fig. 2).

Oral administration of bFGF-CS23 to rats with chronic duodenal ulcers over a period of 21 days stimulated a more than ninefold increase in angiogenesis in the ulcer bed and significantly accelerated ulcer healing compared with untreated animals or with animals treated with optimum doses of the H2-blocker cimetidine.[28,29] Gastric acid and pepsin levels were unaffected by bFGF-CS23 when the growth factor was administered as a single dose. When it was administered for 3 weeks, the volume of gastric juice and acid concentration were significantly increased. These results led to the question, "If the healing of duodenal ulcers can be accelerated without reduction

bFGF

26 70 88 93
——cys————cys———cys—cys— COOH

Acid Resistant
bFGF (CS23)

26 70 88 93
——cys————**ser**———**ser**—cys— COOH

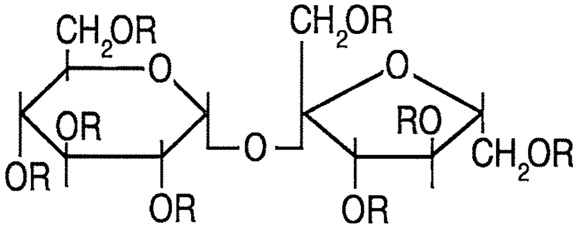
FIG. 2. Structure of bFGF-CS23. Recombinant human bFGF (*upper panel*) was made more acid stable by site-specific mutagenesis in which serine was substituted for the second and third cysteine (*lower panel*).

of gastric acid, what then is the role of gastric acid in duodenal ulceration?"

The widely used anti-ulcer drug Carafate® (sucralfate, *i.e.*, aluminum sucrose octasulfate), provided a clue to this puzzle, because it also accelerates ulcer healing but does not reduce gastric acid. Its mechanism of action is unclear, except that it is known to coat the ulcer bed. Because the chemical structure (Fig. 3) of sucralfate (*i.e.*, a disaccharide with 8 sulfate residues) resembled the repeating disaccharide units of heparin, we tested the affinity of sucralfate for bFGF. We found that sucralfate had a higher affinity to bFGF than heparin did and that it could protect bFGF from degradation by acid.[30]

This surprising result suggested that sucralfate could act by binding endogenous bFGF, protecting it from acid degradation, and delivering it to the ulcer bed. If supporting evidence could be found for this idea, then a new model of the mechanism of ulcer healing could be entertained in which endogenous bFGF, presumably existing in the ulcer bed and in the mucosa, might be critical for ulcer healing because of its angiogenic and other mitogenic properties. In this proposed model, gastric acid would contribute to ulcer formation by continually degrading and inactivating endogenous bFGF, but HCl may not be a primary cause of ulcer formation.

We show here, for the first time, that human and rat gastric and duodenal mucosa contain levels of biologically active bFGF. Basic fibroblast growth factor is present also in the duodenal ulcers of rats. We further demonstrate that oral administration of sucralfate elevates the level of available endogenous bFGF in the ulcer bed. To explain these findings, we propose a unifying hypothesis that suggests that antacids, H2-blockers, and sucralfate act through an FGF-dependent pathway to accelerate the healing of duodenal ulcers. Oral administration of bFGF thus may be considered as a natural form of replacement therapy to achieve accelerated healing of duodenal ulcers.



Sucralfate (Aluminum Sucrose Octasulfate)	R: $SO_3Al(OH)_2$
SOS (Sucrose Octasulfate)	R: SO_3

FIG. 3. Structure of sucralfate. Sucralfate (aluminum sucrose octasulfate) is poorly soluble compared with its soluble counterpart, potassium sucrose octasulfate (SOS).

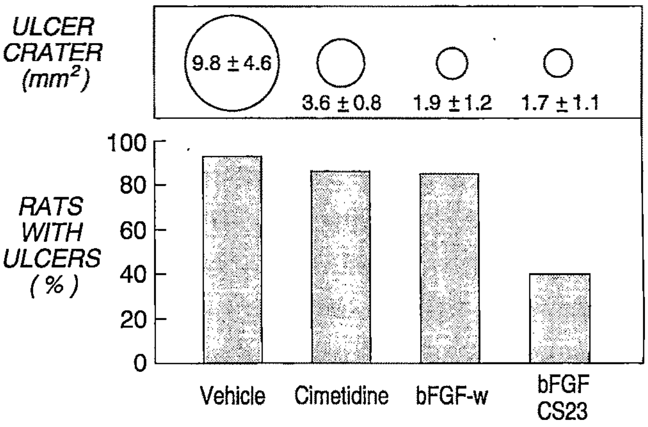


FIG. 4. Effect of orally administered bFGF on the healing of chronic duodenal ulcers. Duodenal ulcers were induced by cysteamine as reported by Szabo *et al.*[41] six rats per bar. Rats received bFGF-w or bFGF-CS23 by oral gavage, 100 ng/100 g twice daily, or vehicle alone, or cimetidine 10 mg/100 g twice daily. The rats were killed at 21 days, the ulcer crater was measured in two dimensions, and the area was calculated by the ellipse formula. The number of residual ulcers was also recorded. At the beginning of the experiment, all rats had penetrating or perforating ulcers deep into the muscularis propria. Redrawn from data in Szabo *et al.*[28]

Oral bFGF-CS23 Is Found to Accelerate Duodenal Ulcer Healing

Chronic duodenal ulcers were produced in rats by feeding cysteamine-HCl® (25 mg/100 g) by intragastric gavage for 3 doses on the first day, using a previously reported method.[31] On the third day, a laparotomy was carried out in all rats, and only those with ulcers deep into the muscularis propria or with penetrating or perforating ulcers were employed in the study.[28,29]

Rats received bFGF-CS23 100 ng/100 g by intragastric gavage twice daily for 21 days. A second group received wild-type bFGF (bFGF-w) that had not been mutagenized for acid stability. A third group received an optimum dose of cimetidine (10 mg/100 g), also by gavage twice daily. Control rats received vehicle alone (sodium citrate buffer 50 nmol/L, pH 7.0). There were six rats per group, and the experiment was repeated four times over a year.[28,29,32]

Treatment with bFGF-CS23 reduced the mean ulcer size (area in square millimeters) down to 17% of untreated controls at 3 weeks ($p = < 0.01$), whereas bFGF-w decreased ulcer size down to 23% of controls ($p = <0.05$) (Fig. 4). Cimetidine decreased ulcer size to 38% of untreated controls, an effect that was only at the margin of statistical significance. The number of rats with residual ulcers at 21 days was: vehicle, 93%; cimetidine, 86%; bFGF-w, 85%; and bFGF-CS23, 40%. Only in rats treated with bFGF-CS23 mutein was the incidence of residual ulcers significantly reduced. The potency of bFGF-CS23 was more than one million times greater than cimetidine on a weight-for-weight basis.

Histologic studies of duodenal ulcers in control rats disclosed a solitary ulcer crater with sparse granulation tissue, necrotic debris, and inflammatory cells. In contrast, animals treated with bFGF-CS23 had completely healed their ulcers or had a small residual ulcer crater with prominent neovascularization. Morphometric analysis of blood vessels stained with antibody to factor VIII-related antigen, a specific marker for vascular endothelial cells, demonstrated a greater than ninefold increase in the number of microvessels in rats treated with bFGF-CS23 compared with animals treated with vehicle alone (Table 1). Rats treated with bFGF-w showed approximately a twofold increase in the number of microvessels compared with rats receiving the vehicle alone. Thus the bFGF-CS23 mutein was superior to the natural form of bFGF in both stimulation of angiogenesis in the ulcer bed and acceleration of ulcer healing.

A single dose of bFGF-CS23 had no effect on gastric acid or pepsin levels. Chronic administration of bFGF-CS23 for 3 weeks increased the volume of gastric juice and acid concentration. Similarly pepsin outputs were increased after 3 weeks of treatment with bFGF-CS23. Interestingly bFGF-CS23 effectively accelerated healing of duodenal ulcers despite the high levels of luminal acid and pepsin. This ability of an angiogenic growth factor to accelerate healing of duodenal ulcers in the presence of high gastric acid and pepsin led us to rethink the role of acid in the pathogenesis of duodenal ulceration. Although most conventional anti-ulcer therapy depends on reduction of gastric acidity by drugs or surgical procedures, sucralfate does not reduce gastric acidity. We therefore directed our attention to elucidating the mechanism of action of sucralfate.

Sucralfate Binds to Fibroblast Growth Factor and Protects It from Acid Degradation

Sucralfate is a basic aluminum salt of sucrose octasulfate that is widely used for the treatment of gastric and duodenal ulcers.[33] This anti-ulcer action is achieved without decreasing gastric acidity.[33] The ability of sucralfate to coat an ulcer bed has been thought to be its principal mode of action. The active components of the molecule seem to be sulfate and sucrose octasulfate.[34] Based on our previous reports that other sulfated saccharides such as heparin[25,35] and beta-cyclodextrin tetradecasulfate[36] have high affinity for fibroblast growth factors, and because the structure of sucrose octasulfate (Fig. 3) resembles the repeating disaccharide structure of heparin, we tested the affinity of sucralfate and sucrose octasulfate for bFGF.

TABLE 1. *Quantification of Vascularization in the Ulcer Bed Compared With Remote Submucosa*

Treatment	No. Microvessels/mm^2 Ulcer Bed	No. Microvessels/mm^2 Submucosa Remote From Ulcer
Vehicle	9.9 ± 3.5	40.0 ± 3.0
bFGF-w	17.9 ± 4.3	43.5 ± 3.2
bFGF-CS23	92.0 ± 5.9	34.2 ± 3.6

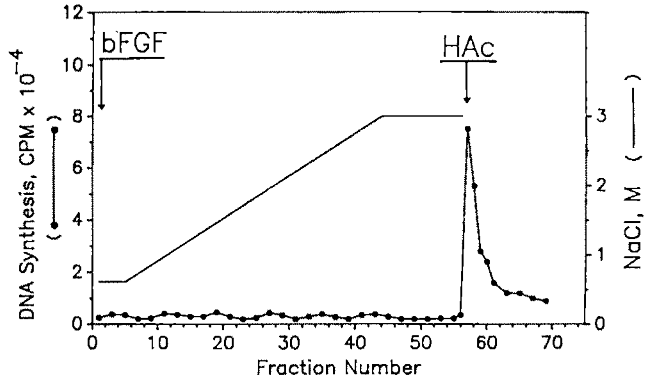


FIG. 5. Elution of bFGF from a sucralfate-Sepharose column by acetic acid. A sucralfate-Sepharose column was prepared by mixing 0.1 g of sucralfate and 0.8 ml (bed volume) of Sepharose CL-6B (Pharmacia) in Tris buffer with 0.6 M NaCl; 2 µg of bFGF in 0.2 ml of saline containing 0.1 mg of BSA was loaded onto the sucralfate column. The column was rinsed with 10 ml Tris buffer and 0.6 M NaCl and eluted consecutively at a flow rate of 15 ml/hr: (1) A gradient of NaCl (80 ml) from 0.6 M to 3.0 M, (2) 20 ml of 3 NaCl, and (3) 30 ml of 1.5 M acetic acid (pH 2.2). Fractions of 2 ml were collected. Aliquots from each fraction were diluted, neutralized with Tris buffer, and analyzed for growth factor activity. The bFGF could not be eluted from a sucralfate-Sepharose column even with 3.0 M NaCl. However, biologically active bFGF was eluted by 1.5 M acetic acid.

Our previous studies showed that bFGF adhered tightly to heparin immobilized on Sepharose beads and could be eluted with a high concentration of NaCl of approximately 1.5 M.[25] In the present experiment, however, when insoluble granules of sucralfate (0.1 g), were mixed with 0.8 ml Sepharose beads to form a chromatographic column and bFGF was loaded onto the column, the peptide bound so avidly to sucralfate that it could not be removed by elution with up to 3 mol/L NaCl (Fig. 5). Thus sucralfate has a higher affinity for bFGF than heparin. Nevertheless bFGF was recovered from this column by elution with acetic acid 1.5 mol/L (pH 2.2). Acetic acid dissolved the sucralfate and bFGF came out in the acidic solution. When this eluate was neutralized with TRIS buffer, diluted 20-fold, and added directly to cultures of 3T3 fibroblasts, the eluted bFGF was highly mitogenic for these cells and retained its full biologic activity. Thus although bFGF is normally degraded and inactivated by acid conditions (see Fig. 1), sucralfate protects bFGF from stronger acid conditions than would normally be found in the stomach (pH 2.2). Water-soluble sucrose octasulfate also protected the biologic activity of bFGF against acid degradation (Fig. 6). If bFGF was denatured by acid conditions before incubation with potassium sucrose octasulfate, however, bFGF biologic activity could not be recovered by addition

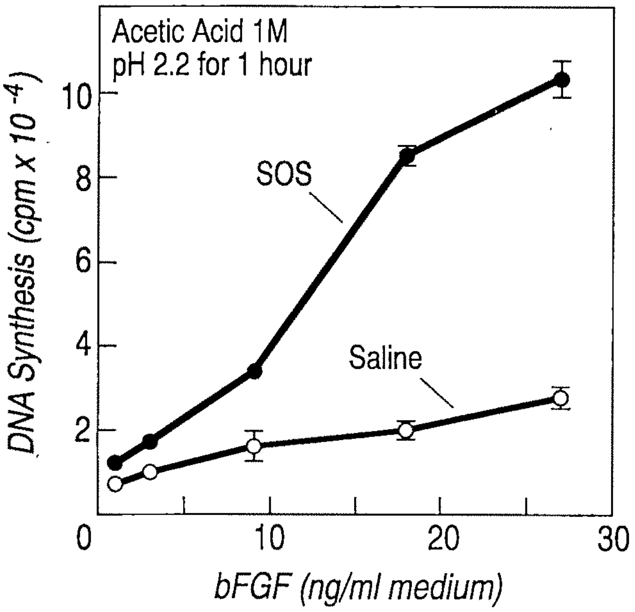


FIG. 6. Stabilization of bFGF by potassium sucrose octasulfate under acidic conditions. The bFGF (0.25 μg/60 μL) was incubated at pH 2.2 with 1 M acetic acid in the presence of (●) and absence of (O) 2 mg potassium sucrose octasulfate at 37 C for 1 hour. At the end of incubation, bFGF samples were diluted and analyzed for their ability to stimulate DNA synthesis on 3T3 fibroblasts.

of the sucrose octasulfate to the treated samples. This indicates that the mechanism of bFGF protection by sucrose octasulfate probably does not involve a renaturation process. Sucrose octasulfate (up to 200 μg/mL) neither increased nor decreased the mitogenic effect of bFGF under neutral pH conditions.

Although these results do not indicate whether the sucrose octasulfate is dissociated from bFGF, such dissociation may not be necessary for the maintenance of biologic activity of bFGF. It is probable that the small sulfated disaccharides attach themselves to specific domains along the full length of the bFGF peptide and protect it from degradation by some as yet unknown mechanism. Nevertheless this protective property of sucralfate for bFGF strongly suggested that if bFGF was naturally present in the stomach, a novel mechanism of action could be assigned to sucralfate.

Evidence that Endogenous bFGF Is Present in the Rat and Human Stomach and Duodenum

Growth Factor Activity in Gastric Mucosa

To determine if any growth factor activity could be extracted from the rat gastric mucosa, sucrose octasulfate was used as the extraction solution. The glandular mucosa of rat stomachs was excised, weighed, and incubated in either saline or sucrose octasulfate (Fig. 7). After centrifugation, an aliquot of the supernatant was assayed for mitogenic activity on 3T3 fibroblasts. Sucrose octasulfate efficiently extracted potent mitogenic activity from the rat gastric mucosa.

The Growth Factor Extracted from Gastric Mucosa Contains bFGF

To demonstrate that this extractable growth factor activity contained biologically active basic fibroblast growth factor (bFGF), glandular mucosa from the rat stomach was extracted in 2 mol/L NaCl and purified by heparin-affinity chromatography. An eluted fraction that coincided with bFGF then was confirmed to contain bFGF by Western blot analysis (see Fig. 9 legend for method). A similar extraction procedure and Western blot analysis was carried out on human gastric mucosa obtained from

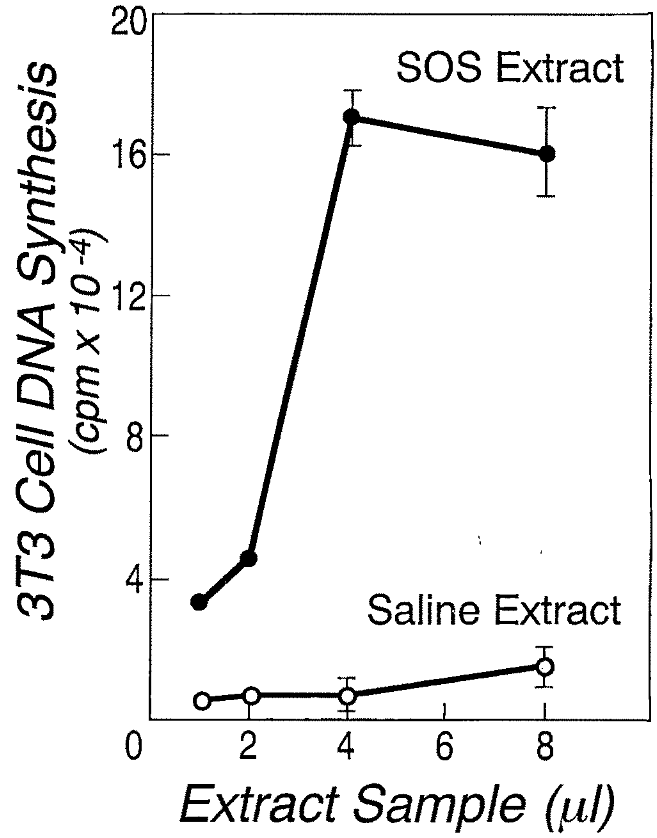


FIG. 7. Elution of growth factor activity from gastric mucosa by sucrose octasulfate (SOS). Sixteen normal rats were killed, and the glandular mucosa of the stomach was excised. The stomachs were randomly divided into two groups. Eight stomachs were individually placed in a volume of either saline or sucrose octasulfate (SOS, 10 mg/ml) to make a weight/volume ratio of 1 g stomach/5 ml solution. Each stomach was then incubated for 12 hours at 4 C with gentle mixing, the extractant centrifuged at 3000× *g* for 30 minutes, and the supernatant assayed on 3T3 fibroblasts for DNA synthesis in a dose-dependent manner. The open circles represent stomachs extracted only in saline. The closed circles show stomachs extracted by SOS. Each point represents eight individual stomachs. Each sample was assayed on 3T3 fibroblasts in triplicate (p = <0.001). Sucrose octasulfate efficiently extracts mitogenic activity from the rat gastric mucosa.

the pyloric antrum. This also confirmed the presence of bFGF.

Evidence that Endogenous bFGF Is Present in the Ulcer Bed and Is Increased by Orally Administered Sucralfate

To determine if the ulcer bed *per se* contained bFGF, rats with penetrating duodenal ulcers were treated for 7 days with either oral sucralfate by gavage or with vehicle alone (Fig. 8). The rats were killed and sucralfate was gently removed from the ulcer bed. The ulcer bed was excised, weighed, and extracted in 2 mol/L NaCl for 12 hours. After centrifugation, the supernatant was diluted and applied to a heparin-affinity column. The eluted peak of bFGF was assayed for mitogenic activity on 3T3 fibroblasts. Basic fibroblast growth factor also was extracted from proximal stomach and duodenum. Figure 8 demonstrates a significant increase in extractable bFGF in the ulcer bed of sucralfate-treated animals compared with the untreated rats (p = <0.001). Moreover untreated ulcers contained significantly more bFGF than normal mucosa from stomach or duodenum. In Figure 9A, Western blot analysis of bFGF extracted from the ulcer beds and purified by heparin-affinity chromatography confirms its identity to pure recombinant bFGF having a molecular weight of 18,000. Lane 1 shows human recombinant bFGF. Lane 2 shows bFGF extracted from untreated ulcer beds. Lane 3 shows bFGF extracted from ulcer beds after treatment with sucralfate. The band in lane 3 is significantly stronger than the band in lane 2. This is consistent with the much higher level of growth factor activity observed in the sucralfate-treated ulcer bed in Figure 8.

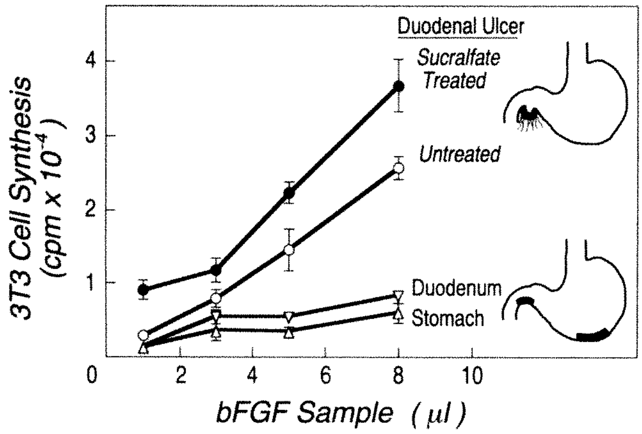


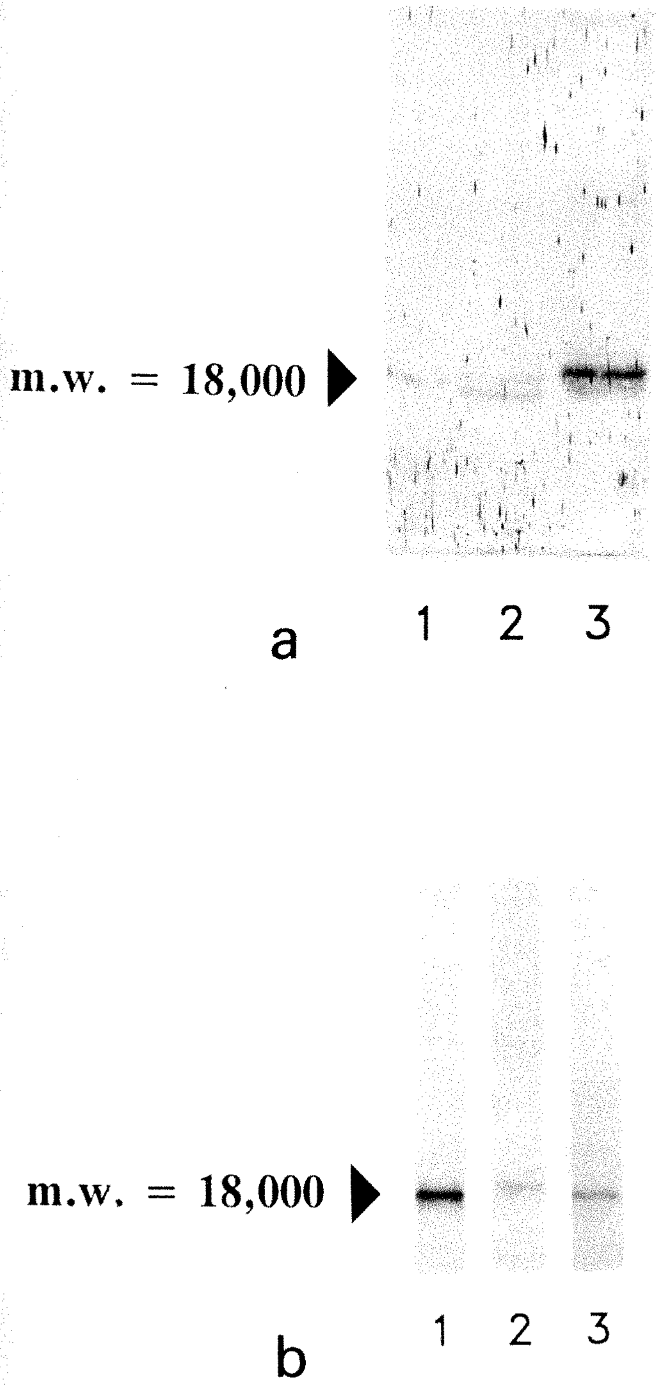
FIG. 8. Effect of sucralfate on endogenous bFGF in duodenal activity from the rat gastric mucosa. Duodenal ulcers were induced in 20 rats by cysteamine. Ten rats received oral sucralfate by gavage, 20 mg/100 g, twice daily for 7 days. Ten rats received the vehicle solution (water) by gavage. Both groups of rats were killed on day 8. The sucralfate was gently washed away from where it had coated the ulcer bed, one gram of stomach was then incubated in 5 ml NaCl 2.0 M (*e.g.*, 500 mg stomach in 2.5 ml NaCl), plus 10 mM Tris at pH 7.0 to extract FGF from the tissues at 4 C for 12 hours. The extractant was centrifuged at 3000× *g* for 30 minutes, and the supernatant was diluted fourfold with 10 mM Tris buffer to lower the salt concentration. The diluted supernatant was then applied to a heparin-affinity column. The column was rinsed with 0.6 M NaCl and eluted with NaCl 2.0 M, and the fractions were assayed for mitogenic activity on 3T3 fibroblasts. All of the mitogenic activity was in the 2.0 M NaCl fraction. For control subjects, proximal portions of stomach and duodenum were also sampled and weighed in the same way and analyzed by heparin-affinity chromatography. There is a significant increase of bFGF in the ulcer bed of sucralfate-treated animals compared with the untreated animals (p = <0.001). Furthermore, the ulcer beds of untreated animals had significantly more bFGF than normal duodenal or stomach mucosa (p = <0.005). Each point represents the mean triplicate 3T3 assay from an aggregate of 10 animals. Error bars SEM. The identity of the bFGF in the ulcer bed and in the mucosa was confirmed by Western blot analysis (Fig. 9).

Immunohistochemical Localization of bFGF in the Gastric and Duodenal Mucosa

To determine the localization of bFGF in the stomach and duodenum, histologic sections of these tissues were stained with two different antisera specific for bFGF (Fig. 10). Figure 10 (upper panel) shows intense staining of bFGF in the mucosa and submucosa of the rat stomach. Figure 10 (lower panel) is a control histologic section in which the bFGF antibody was blocked with excess antigen. Almost no staining is observed. Rat duodenum and colon demonstrated similar mucosal and submucosal localization of bFGF (not shown).

Sucralfate Stimulates Angiogenesis

To determine if sucralfate could stimulate angiogenesis, sterile sucralfate pellets were implanted in the rabbit cornea (Fig. 11A, B). Sucralfate alone induced the formation of new capillary blood vessels after a delay of 4 to 6 days. When sucralfate was mixed with bFGF, intense angiogenesis was observed as early as 2 days. Angiogenesis was similarly induced by sucralfate or sucralfate-bFGF pellets implanted on the chorioallantoic membrane of the chick embryo and in the subcutaneous dorsal air sac of rats (not shown). Figure 11A shows that a sucralfate pellet of 10 mg containing 5 µg bFGF implanted in the rabbit cornea induced new blood vessels that attained a maximum length of 2.8 times the length of vessels induced by sucralfate alone (Fig. 11B). More striking was the fact that the sucralfate-bFGF pellets induced neovascularization over the full circumference of the cornea. In contrast sucralfate alone induced only a narrow sector of neovascularization. Even in the presence of neovascularization, the corneas remained clear and transparent. Histologic sections after 12 days showed virtually no inflammatory cells in the corneas. Sucralfate-bFGF mixtures induced the most intense and prolonged angiogenesis of any noninflammatory angiogenic compound that has been tested in the cornea. By approximately 25 to 30 days, the sucralfate pellets had begun to disappear. We speculate that implanted sucralfate pellets extract and bind available



FIGS. 9A and B. Western blot analysis of bFGF in rat and human stomach. (A) The extractions from the sucralfate-treated ulcers and the untreated rat ulcers described in Fig. 8, were then purified on heparin-affinity chromatography as previously described by Shing *et al.*[25] Fractions eluted from the heparin-affinity column at approximately 1.5 M NaCl, which coincides with elution for pure bFGF, were then analyzed by Western blot analysis using a polyclonal antibody produced against the internal synthetic peptide of bFGF.

Briefly, the active fraction from heparin-affinity chromatography was dialyzed against water (changing twice overnight) in 4 L of water and then lyophilized. The lyophilizate was applied to a 15% SDS polyacrylamide electrophoresis gel, as described by Lemmeli. The SDS gel was then transferred to a nitrocellulose paper that was then incubated with a polyclonal antibody produced in rabbits against the synthetic internal sequence of human bFGF. This blot was then incubated with a secondary antibody (goat anti-rabbit IgG) conjugated to alkaline phosphatase. The blot was then stained for alkaline phosphatase.

In (A), Lane 1 shows bFGF (18,000 molecular weight). Lane 2 shows bFGF extracted from the duodenal ulcer of an untreated rat. Lane 3 shows bFGF isolated from the ulcer bed of a sucralfate-treated rat.

In (B), Lane 1 shows bFGF. Lane 2 shows bFGF isolated from rat stomach mucosa. Lane 3 shows bFGF isolated from human stomach mucosa (discarded at the time of gastrectomy).

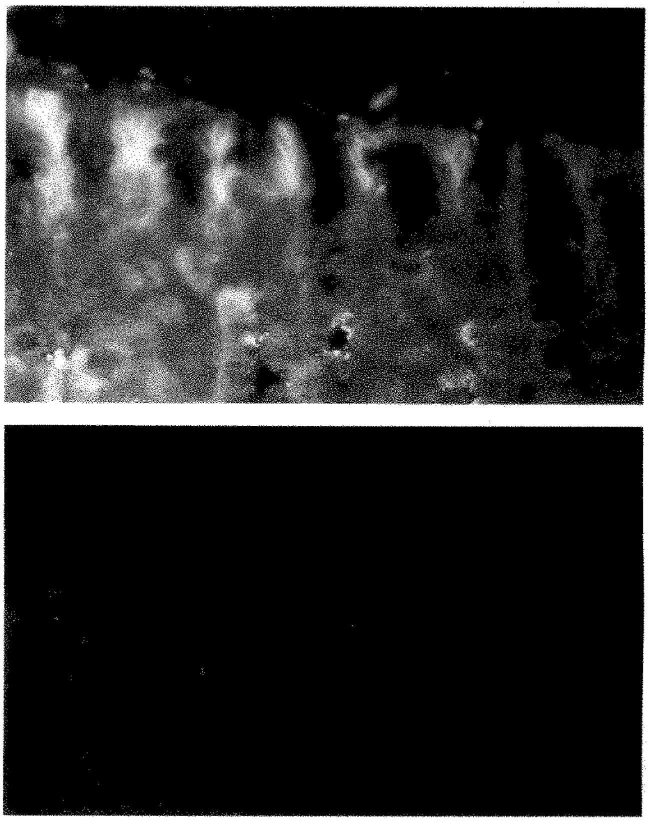
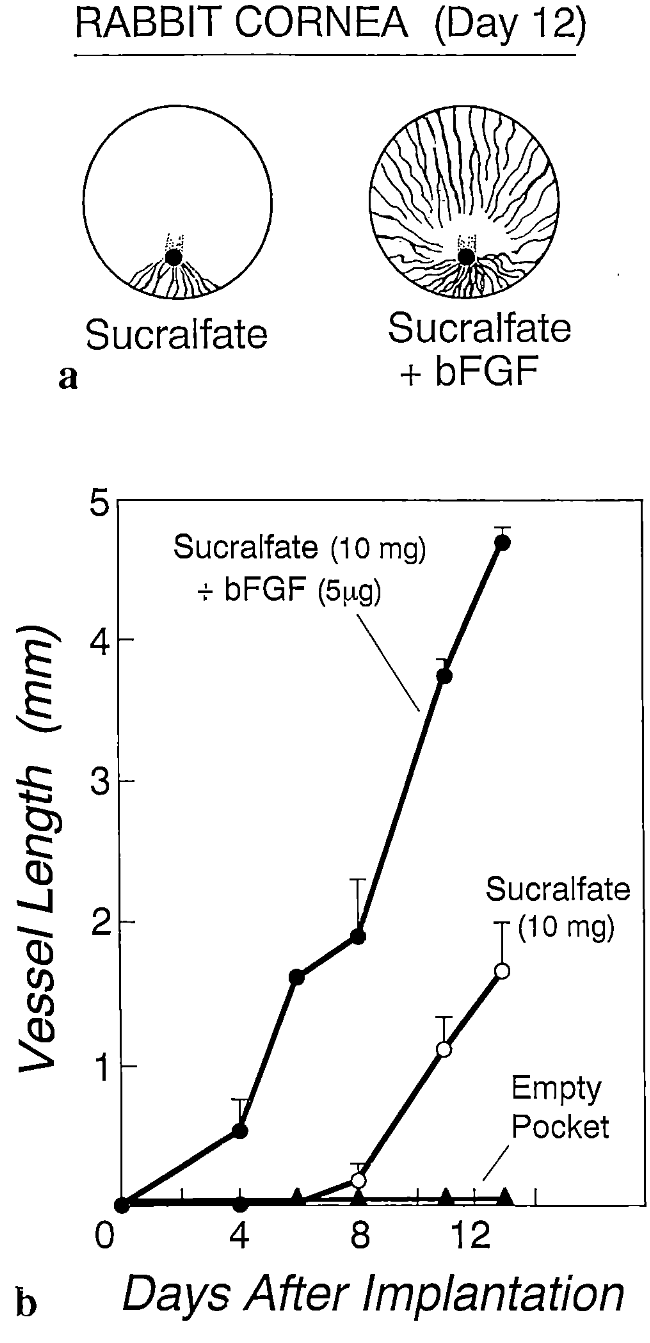


FIG. 10. Demonstration of bFGF in rat gastric mucosa by immunohistochemical staining. Female Sprague-Dawley rats 200–250 g were killed with sodium pentobarbital, and sections of the forestomach, glandular stomach, duodenum, and colon were obtained. The tissues were fixed with 4% paraformaldehyde cryoprotected in 30% sucrose. Immunohistochemical staining was performed with a murine monoclonal antibody directed against the N-terminal sequence of human bFGF (gift from Takeda Chemical Industries, Ltd., Osaka, Japan). This antibody reacts with bFGF but not aFGF on Western blots and not with other proteins. The cryostat sections were fixed for 10 minutes with 10 mM NaCl in PBS. Tissue sections were washed three times with PBS, permeabilized in 0.2% Triton, and then incubated with normal goat serum for 60 minutes. The blocking buffer was then drained off and the sections were incubated for 12 hours with the primary antibody of 1:400 dilution.

Control sections were stained with antisera preincubated in the presence of an excess (50×) of the immunizing peptide for 3 hours 37 C or with nonimmune serum alone. The sections were washed with PBS and incubated for 3 hours with secondary antisera (a fluorescein-labeled goat anti-murine IgG). The tissue sections were washed, mounted in an antibleaching agent, and examined by fluorescence microscopy.

Intense staining (*upper panel*) for bFGF in the gastric mucosa taken from a region in the rat antrum. Absence of staining *lower panel* when anti-bFGF antibody was blocked by excess antigen. Duodenal and colon mucosa also demonstrated significant staining for the presence of bFGF. A similar staining pattern was obtained using a polyclonal antibody directed against human bFGF (gift from Dr. Patricia D'Amore, Harvard Medical School).



FIGS. 11A and B. Angiogenic activity of sucralfate and sucralfate complexed with bFGF. (A) Drawing from Ektachrome photographs. Pure sucralfate was sterilized by boiling for 1 minute. A portion of the sucralfate was complexed with bFGF (10 mg sucralfate plus 5 μg of bFGF by gentle mixing for 1 hr at room temperature). A 10-mg pellet of sucralfate was implanted into a rabbit corneal pocket 2 mm from the limbal edge by a technique previously reported.[1]

Sucralfate complexed to bFGF was implanted in the opposite cornea or in the cornea of a different rabbit. Five eyes were used from each group. (B) Mean maximum vessel length and SEM measured at intervals from the time of implantation.

Sucralfate plus bFGF induced intense angiogenesis around the entire cornea without inflammation or edema. Sucralfate alone had no effect until about day 6, after which neovascularization appeared in a narrow sector closest to the implanted pellet.

bFGF that is produced in the local tissue. The bound bFGF then is protected from degradation and slowly released as the sucralfate gradually dissolves. In contrast sucralfate that is precomplexed with bFGF acts as a slow release depot for the biologically active peptide, leading to the induction of more intense angiogenesis. Preliminary data from the chick embryo support this notion.

A Unifying Hypothesis for the Mechanism of Accelerated Ulcer Healing

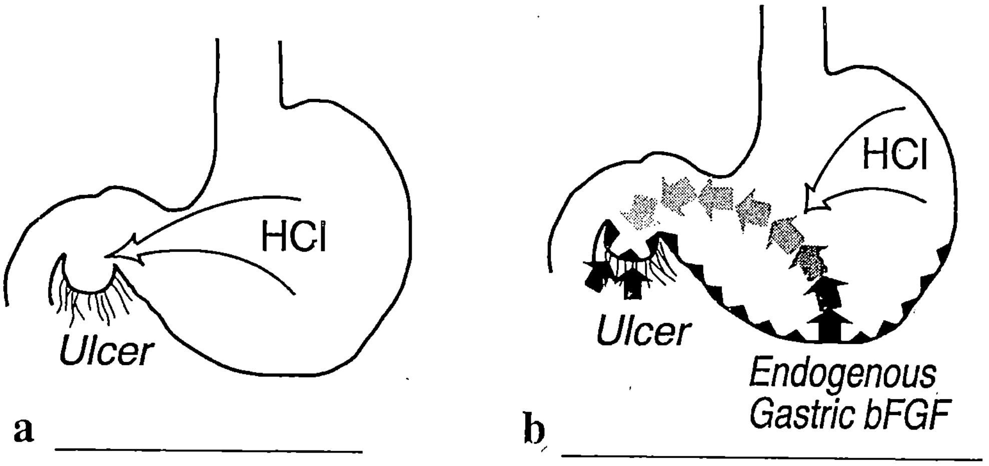
These experiments suggest a model in which conventional anti-ulcer therapies may operate through a common mechanism. In this model naturally occurring endogenous bFGF is produced by gastric and duodenal mucosa as well as within the ulcer bed (possibly by macrophages and mast cells, etc.) (Fig. 12A, B). This peptide may be necessary for ulcer healing by virtue of its angiogenic activity as well as its mitogenic activity for fibroblasts, smooth muscle, and epithelium. This endogenous bFGF, however, is highly susceptible to continuous degradation and inactvation by gastric acid.

According to this hypothetical model, anti-ulcer therapies that reduce acid (*e.g.*, antacids, H2-antagonists, surgical procedures) would retard degradation of endogenous bFGF (Fig. 12C, D). Sucralfate therapy would protect bFGF from acid degradation, without affecting gastric acidity *per se* (Fig. 12E). Oral administration of acid-stable bFGF (*e.g.*, bFGF-CS23) could be considered as a form of replacement therapy (Fig. 12F). Such therapy would overcome the problem of steady degradative losses of endogenous bFGF.

The role of hydrochloric acid in the pathogenesis of peptic ulcer would, in the context of this model, depend on the extent to which HCl degraded endogenous bFGF.

The Effect of Cimetidine on bFGF Levels in the Ulcer Bed

One important prediction that arises from this hypothetical model is that cimetidine therapy should elevate available levels of bFGF in the ulcer bed as a result of a significant reduction in gastric acidity. To test this prediction, rats with penetrating duodenal ulcers induced by Cysteamine were treated with high doses of cimetidine by intragastric gavage (20 mg/100 g, twice daily) to maximally raise gastric pH. Control rats received vehicle alone. The animals were killed after 5 days, and the gastric pH was measured. The ulcer beds were excised and the bFGF in the ulcer bed was extracted, purified, and quantitated (as described in the legend of Fig. 8). The cimetidine-treated animals had a pH range of 6.4 to 7.2, and their ulcer beds contained significantly higher levels of biologically active bFGF than did those of untreated animals (pH 3.4–4.3)



HCl = Primary
(Conventional
Mechanism)

🡅 FGF = Primary
HCl degrades FGF
(Proposed mechanism)

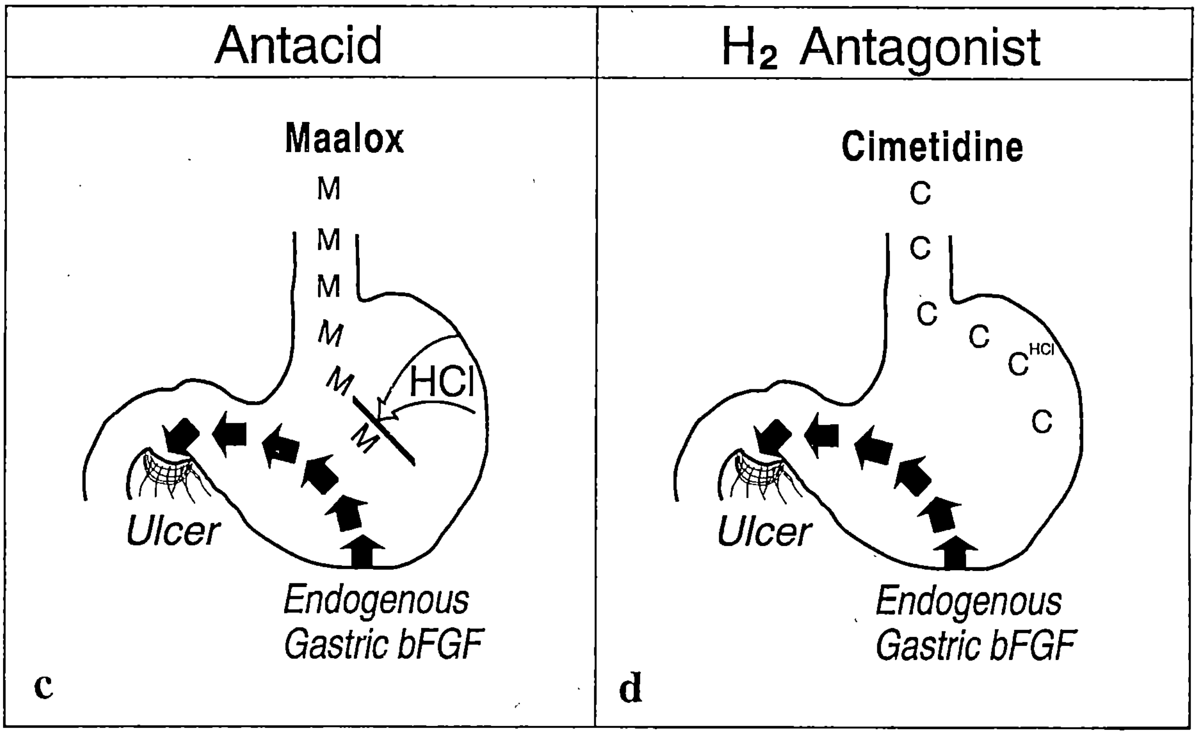


FIG. 12. Models of ulcer formation and anti-ulcer therapy. (a) A conventional model in which HCl is believed to directly cause duodenal ulcers. (b) A proposed mechanism in which endogenous bFGF in the stomach and duodenum may act to prevent ulceration unless this peptide is excessively degraded by HCl. (c) A hypothetical model of anti-ulcer therapy in which neutralization of HCl permits increased availability of endogenous bFGF in the ulcer bed. (d) A hypothetical model of anti-ulcer therapy in which an H2 antagonist decreases secretion of HCl, thus interfering with degradation of bFGF in the gastric mucosa in the ulcer bed. (e) A hypothetical model of anti-ulcer therapy in which sucralfate binds and protects available endogenous bFGF without affecting levels of HCl. (f) A hypothetical model of anti-ulcer therapy by oral delivery of an angiogenic peptide that has been made acid resistant (bFGF-CS23). In this model, exogenous bFGF can be considered as replacement therapy for endogenous bFGF undergoing degradation by HCl.

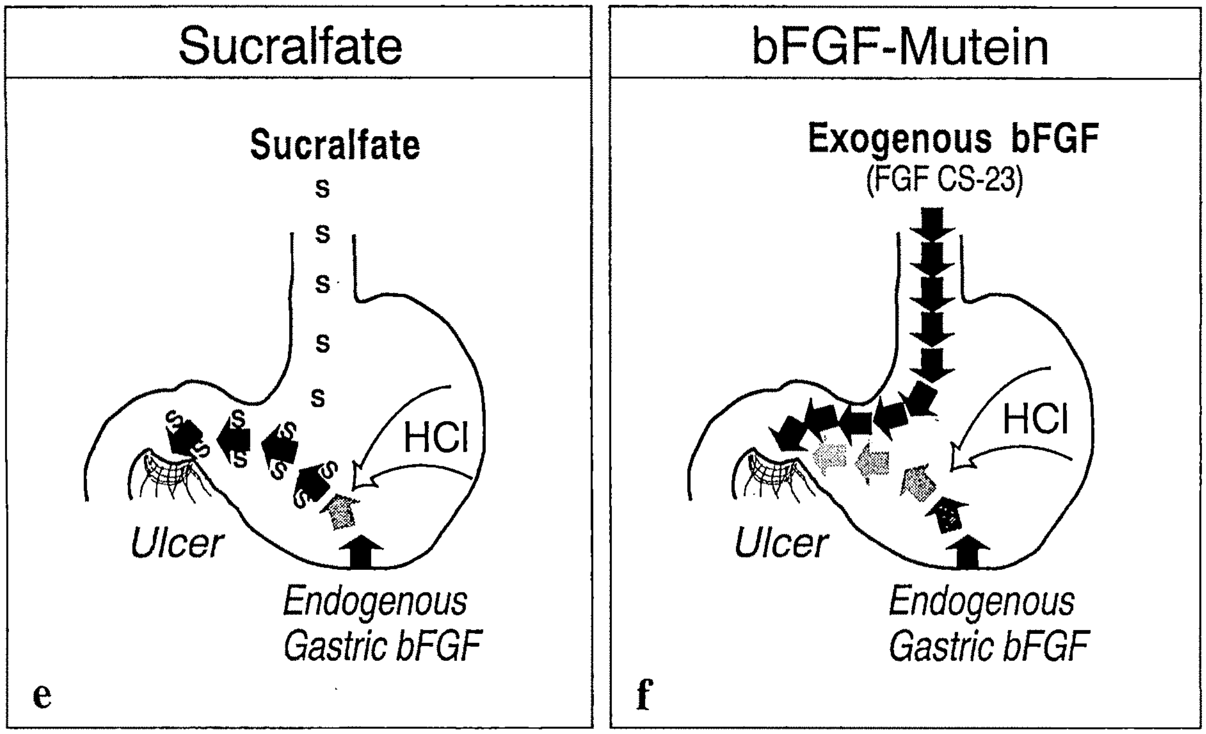


FIG. 12. (*Continued*)

($p < 0.001$), (Fig. 13). These results suggest that cimetidine anti-ulcer therapy may be FGF dependent.

Discussion

These studies show that oral administration of an acid-stable form of bFGF (bFGF-CS23) stimulates angiogenesis in the ulcer bed and significantly accelerates the healing of chronic duodenal ulcers in rats. Although our studies do not prove a causal relationship between enhanced angiogenesis and accelerated ulcer healing, neovascularization is a critical event in wound healing, and peptic ulcers are not dissimilar from chronic wounds. Furthermore bFGF is a growth factor for fibroblasts, smooth muscle cells, and epithelium.[26] Its ability to stimulate proliferation of these cells also could contribute to accelerated ulcer healing.

These experiments demonstrate for the first time that biologically active bFGF is present in human and rat gastric and duodenal mucosa, and also in the bed of duodenal ulcers in rats. Our studies do not define which cells are the source of bFGF in the stomach or duodenum. A recent report suggests that bFGF may be present also in normal human colon, but the method of detection was by immunostaining alone; biologic activity was not measured.[37]

Because sucralfate is now shown to be capable of binding and protecting bFGF from acid degradation, this finding may explain its mechanism of action. When this result is taken together with the finding that both sucralfate and cimetidine elevate levels of endogenous bFGF in the stomach and in the ulcer bed, it appears that the anti-ulcer properties of these drugs may be FGF dependent. We have extended this concept to construct a hypothetical model of ulcer formation and ulcer therapy in which endogenous bFGF is continually produced by gastric and duodenal mucosa, and may play a protective or preventive role in ulcer formation. Production of endogenous bFGF is balanced, however, by continuous inactivation of the peptide because of its acid sensitivity. Once an ulcer has formed, bFGF also appears to be produced in the ulcer bed, and may be a possible contributing factor in cases of spontaneous healing.

The preventive role of bFGF in ulcer formation is speculative, and we currently have no direct evidence for it. It remains to be seen whether endogenous bFGF eventually will be considered as a component of the cytoprotective mechanism in gastric mucosa.[38–40]

We wish to emphasize that the hypothesis proposed here attempts to provide a unifying mechanism for the anti-ulcer effect of H2-antagonists, antacids and sucralfate, based on our data. This hypothesis does not take into account other anti-ulcer therapies, such as prostaglandins,[41] and it may not apply to stress ulcers.[42]

Furthermore bFGF is not the only endogenous growth factor likely to be important in healing of duodenal ulcers or in their prevention. Epidermal growth factor (EGF),

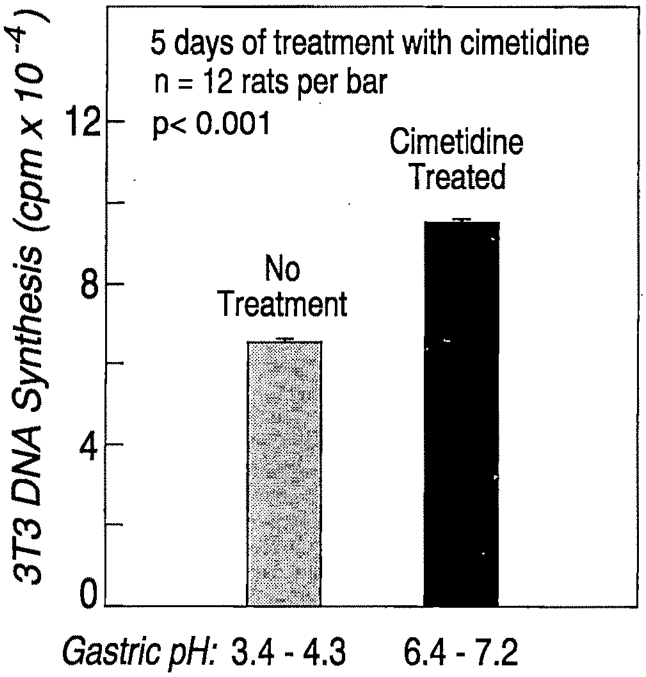


FIG. 13. Effect of cimetidine therapy on endogenous gastric bFGF. Twenty four rats with chronic duodenal ulcers induced by cysteamine were divided randomly into two groups of 12 rats each. The first group received no treatment except for gavage with vehicle (water) twice daily. The second group received cimetidine, 20 mg/100 g by intragastric gavage twice daily for 5 days. All animals were killed on day 6, and the pH of the gastric juice was measured. The untreated animals had a pH range of 3.4 to 4.3 compared with the cimetidine-treated animals, which had a pH of 6.4 to 7.2. The ulcer beds were excised, and the bFGF in the ulcer bed was analyzed as described in the legend of Figs. 8 and 9. The extracted bFGF was analyzed for DNA synthesis activity on the 3T3 fibroblasts. Cimetidine-treated animals had significantly higher levels of biologically active bFGF in the ulcer bed than untreated animals (p = <0.001).

found in saliva, can accelerate healing of experimental ulcers and is about as effective as cimetidine.[43–45] It also reduces gastric acid.[46] Transforming growth factor-alpha (TGF-α), shares about 35% amino acid sequence homology with EGF and binds to the same cell surface receptor as EGF. Transforming growth factor-alpha also inhibits gastric acid secretion.[47] It is angiogenic,[48] although it may be less potent than bFGF. Expression of mRNA for TGF-α has been found in the gastric mucosa of guinea pig, rat, and dog, and also in the gastric mucosa of patients undergoing gastric resection for peptic ulcer or for cancer.[49]

Studies of healing wounds show a cascade of growth factors released at first by platelets (*i.e.*, platelet-derived growth factor and TGF-β), and later by macrophages and other cells that enter the wound. If the principles of peptic ulcer healing are similar to the general rules of chronic wound healing, then one might predict that multiple endogenous growth factors may be cooperating to bring about spontaneous healing of a duodenal ulcer. If and when such factors are uncovered, they also may form the basis of novel therapies when used together with an angiogenic peptide like bFGF-CS23, or separately, or as adjuncts to conventional anti-ulcer therapy. It is also possible that sucralfate-bFGF complexes may be more effective than either compound alone. It remains to be seen whether such a complex could accelerate the healing of ulcers in other parts of the gastrointestinal tract, such as in the colon. We have shown previously that bFGF-CS23 administered per rectum can accelerate healing of experimental colonic ulcers in rats.[50]

Our demonstrations that an orally delivered angiogenic peptide can accelerate ulcer healing, and that a natural form of this peptide already exists in the mucosa, have led to a novel view of ulcer healing and anti-ulcer therapy. This new perspective may well generate fruitful experiments, (*e.g.*, the effect of vagotomy on endogenous bFGF), regardless of the half-life of the hypothetical model itself.

Summary

Oral administration of an acid-stable form of the angiogenic peptide, basic fibroblast growth factor (bFGF-CS23) stimulates angiogenesis in experimental duodenal ulcers and accelerates their healing more potently than cimetidine. Naturally occurring endogenous bFGF is present in the rat and human gastric and duodenal mucosa, but is very sensitive to acid degradation.

Sucralfate and sucrose octasulfate have a high affinity for bFGF and protect bFGF from acid degradation and inactivation. Oral administration of sucralfate elevates local levels of bFGF in the ulcer bed. Sucralfate is also a potent angiogenesis stimulator, apparently on the basis of its ability to stabilize and slowly release locally available bFGF. This may be the mechanism of its anti-ulcer efficacy, because it does not reduce gastric acid.

Cimetidine therapy, which does reduce gastric acid, also significantly increases the level of biologically active bFGF in the ulcer bed. The mechanism here may be related to the longer survival of endogenous bFGF at slightly higher pH.

A unifying hypothesis is proposed to provide a common mechanism for conventional anti-ulcer therapy. In this model, endogenous bFGF plays a central role, and oral administration of exogenous bFGF-CS23 provides a form of replacement therapy as a new approach to the problem of duodenal ulcer.

References

1. Folkman J. Angiogenesis and its inhibitors. *In* DeVita VT Jr, Hellman S, Rosenberg SA, eds. Important Advances in Oncology, Philadelphia: JB Lippincott, 1985; pp 42–62.
2. Folkman J. Angiogenesis. *In* Verstraete M, Vermylen J, Lijnen HR,

Arnout J, eds. Thrombosis and Haemostasis. Leuven: Leuven University Press, 1987; pp 583–596.

3. Folkman J, Klagsbrun M. Angiogenic factors. Science 1987; 235: 442–447.
4. Folkman J, Ingber DE. Angiostatic steroids. *In* Schleimer RP, Claman HN, Oronsky AL, eds. Anti-inflammatory Steroid Action: Basic and Clinical Aspects, Chap. 14. New York: Academic Press, 1989; pp 330–350.
5. Klagsbrun M, Folkman J. Angiogenesis. *In* Sporn MB, Roberts AB, eds. Handbook of Experimental Pharmacology, Peptide Growth Factors and Their Receptors, Part II. Heidelberg: Springer Verlag, 1990; 95:549–586.
6. Weidner N, Semple J, Welch W, Folkman J. Tumor angiogenesis correlates with metastasis in invasive breast carcinoma. N Engl J Med 1991; 324:1–8.
7. White CM, Sondheimer HM, Crouch EC, et al. Treatment of pulmonary hemangiomatosis with recombinant interferon alfa-2a. N Engl J Med 1989; 320(18):1197–1200.
8. Folkman J. Successful treatment of an angiogenic disease. N Engl J Med 1989; 320:1211–1212.
9. Ingber D, Fujita T, Kishimoto S, et al. Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth. Nature 1990; 348(6301):555–557.
10. Robson MC. Peptide growth factors in the treatment of chronic wounds, personal communication, 1991.
11. Knighton DR, Hunt TK, Thakral KK, Goodson WH. Role of platelets and fibrin in the healing sequence: an *in vivo* study of angiogenesis and collagen synthesis. Ann Surg 1982; 196:379–388.
12. Hunt TK, Knighton DR, Thakral KK, et al. Studies on inflammation and wound healing: angiogenesis and collagen synthesis stimulated *in vivo* by resident and activated wound macrophages. Surgery 1984; 96(1):48–54.
13. Leibovich SJ, Ross R. The role of macrophage in wound repair. Am J Pathol 1985; 78:71–100.
14. McGrath MH, Emery JMI. The effect of inhibition of angiogenesis in granulation tissue on wound healing and the fibroblast. Ann Plast Surg 1985; 15:106–119.
15. Broadley KN, Aquino AM, Woodward SC, et al. Monospecific antibodies implicate basic fibroblast growth factor in normal wound repair. Lab Invest 1989; 61(5):571–575.
16. Buntrock P, Jentzsch KD, Heder G. Stimulation of wound healing, using brain extract with fibroblast growth factor formation of granulation tissue. Exp Pathol 1982; 21:46–53.
17. Buntrock P, Jentzsch KD, Heder G. Stimulation of wound healing, using brain extract with fibroblast growth factor (FGF) activity: II. Histological and morphometric examination of cells and capillaries. Exp Pathol 1982; 21:62–67.
18. Davidson JM, Klagsbrun M, Hill KE, et al. Accelerated wound repair, cell proliferation, and collagen accumulation are produced by a cartilage-derived growth factor. J Cell Biol 1985; 100:1219–1227.
19. Sprugel KH, McPherson JM, Clowes AW, Ross R. Effects of growth factors *in vivo:* I. Cell ingrowth into porous subcutaneous chambers. Am J Pathol 1987; 129:601–613.
20. McGee GS, Davidson JM, Buckley A, et al. Recombinant basic fibroblast growth factor accelerates wound healing. J Surg Res 1988; 45:145–153.
21. Davidson J, Buckley A, Woodward S, et al. *In* Barbul A, Pines E, Caldwell M, Hunt TK, eds. New York: Alan R Liss, 1988; pp 63–75.
22. Thomas AK, DiSalvo J, Ortega S, et al. Growth Factors and Their Receptors: Genetic Control and Rational Application. New York: Alan R Liss, 1989; pp 253–258.
23. Greenhalgh DG, Sprugel KH, Murray MJ, Ross R. PDGF and FGF stimulate wound healing in the genetically diabetic mouse. Am J Pathol 1990; 136(6):1235–1246.
24. Steinberg BD, Phillips LG, Hokanson JA, et al. Effect of bFGF on the inhibition of contraction caused by bacteria. J Surg Res 1991; 50:47–50.
25. Shing Y, Folkman J, Sullivan R, et al. Heparin affinity: purification of a tumor-derived capillary endothelial cell growth factor. Science 1984; 223:1296–1298.
26. Gospodarowicz D, Ferrara N, Schweigerer L, Neufeld G. Structural characterization and biological functions of fibroblast growth factor. Endocr Rev 1987; 8:95–114.
27. Seno K, Sasada R, Iwane K, et al. Stabilizing basic fibroblast growth factor using protein engineering. Biochem Biophys Res Commun 1988; 151:701–708.
28. Szabo S, Vattay P, Morales RE, et al. Orally administered FGF mutein: effect on healing of chronic duodenal ulcers in rats. Dig Dis Sci 1989; 34(8):1323.
29. Folkman J, Szabo S, Vattay P, et al. Effect of oral administration of bFGF on healing of chronic duodenal ulcers, gastric secretion and acute mucosal lesions in rats. Gastroenterology 1990; 98(5): 45.
30. Folkman J, Szabo S, Shing Y. Sucralfate affinity for fibroblast growth factor. J Cell Biol 1990; 111(5):223a.
31. Szabo S. Animal model: cysteamine-induced chronic duodenal ulcer in the rat. Am J Pathol 1974; 93:273–276.
32. Szabo S, Folkman J, Vattay P, et al. 1991 (Submitted for publication).
33. Szabo S, Hollander D. Pathways of gastrointestinal protection and repair: mechanisms of action of sucralfate. Am J Med 1989; 86(6A):23–31.
34. Szabo S, Brown A. Prevention of ethanol induced vascular injury on gastric mucosal lesions by sucralfate and its components: possible role of endogenous sulfhydryls. Proc Soc Exp Biol Med USA 1987; 185:493–497.
35. Klagsbrun M, Shing Y. Heparin affinity of anionic and cationic capillary endothelial cell growth factors: analysis of hypothalamus-derived growth factors and fibroblast growth factors. Proc Soc Exp Biol Med USA 1985; 82:805–809.
36. Shing Y, Folkman J, Weisz PB, et al. Affinity of fibroblast growth factors for beta-cyclodextrin tetradecasulfate. Anal Biochem 1990; 185:108–111.
37. Cordon-Carlo C, Vlodavsky I, Haimovitz-Friedman A, et al. Expression of basic fibroblast growth factor in normal human tissues. Lab Invest 1990; 63(6):832–840.
38. Silen W. What is cytoprotection of the gastric mucosa? Gastroenterology 1988; 94(1):232–235.
39. Silen W, Schiessel R, Kivilaasko E. The gastric mucosal barrier and ulceration. Brain Res Bull 1980; 5(1):3–6.
40. Szabo S, Vattay P. Experimental gastric and duodenal ulcers. *In* Hunt RH, ed. Gastroenterology Clinics of North America. Philadelphia: WB Saunders, 1990, pp 67–85.
41. Takeuchi K, Svanes K, Critchlow J, et al. Prostaglandins stimulate and inhibit acid secretion in amphibian fundic mucosa. Proc Soc Exp Biol Med USA 1982; 170(4):398–404.
42. Silen W. The clinical problem of stress ulcers. Clin Invest Med 1987; 10(3):270–274.
43. Olsen PS, Poulsen SS, Therkelson K, Nexo E. Oral administration of synthetic human urogastrone promotes healing of chronic duodenal ulcers in rats. Gastroenterology 1986; 90:911–917.
44. Konturek SJ, Brzozowski T, Dembinski A, et al. Advances in drug therapy of gastrointestinal ulceration. *In* Garner A, Whittle BJR, eds. New York: John Wiley & Sons, 1989; pp 261–273.
45. Konturek SJ. Role of growth in gastroduodenal protection and healing of peptic ulcers. *In* Hunt RH. Philadelphia: WB Saunders, 1990; pp 41–65.
46. Finke U, Rutten M, Murphy RA, Silen W. Effects of epidermal growth factor on acid secretion from guinea pig gastric mucosa: *in vitro* analysis. Gastroenterology 1985; 88:1175–1182.
47. Rhodes JA, Tam JP, Finke U, et al. Transforming growth factor-alpha inhibits the secretion of gastric acid. Proc Natl Acad Sci USA 1986; 83(11):3844–3846.
48. Schreiber AB, Winkler ME, Derynck R. Transforming growth factor-alpha: a more potent angiogenic mediator than epidermal growth factor. Science 1986; 232:1250–1253.
49. Beauchamp RD, Barnard JA, McCutchen CM, et al. Localization of transforming growth factor alpha and its receptor in gastric mucosal cells. Implications for a regulatory role in acid secretion and mucosal renewal. J Clin Invest 1989; 84(3):1017–1023.
50. Satoh H, Takami K, Kato K, et al. Effect of bFGF and its mutein on healing of colonic ulcers induced by N-ethylmaleimide in rats. Gastroenterology 1990; 98(5):45.

DISCUSSION

DR. WILLIAM SILEN (Boston, Massachusetts): Judah Folkman has again stimulated us to reevaluate traditional concepts and in so doing will provoke us to carry out studies to examine further his intriguing proposal.

While his data strongly support the suggestion that basic FGF accelerates the healing of cysteamine-induced ulcers without influencing acid secretion, and also provide a common mechanism by which a variety of agents favorably influence ulcer healing, I would caution, as he has in his manuscript, which he shared with me over a week ago, that the preventive role of endogenous FGF in ulcer formation is speculative and that there is no direct evidence for that at present.

One need only recall that many jump to an analogous erroneous conclusion much too soon with regard to prostaglandins.

This note of caution in no way diminishes or mitigates the importance of his findings.

I have three questions. First because FGF can cause the wound to contract, could you separate for us the effects of wound contraction from those of angiogenesis, or, phrased another way, can you inhibit the angiogenic effects of FGF and still retain the wound contraction effects, and what does that do to ulcer healing?

Secondly what is the relative importance of the other growth factors, such as EGF or TGF, in comparison with the healing effects of FGF?

And finally have you tested FGF against models of ulcers induced by other means?

These and many other questions are now waiting to be answered, and I congratulate Dr. Folkman and his colleagues on a most stimulating paper. Thank you.

DR. JAMES C. THOMPSON (Galveston, Texas): A fellow could make a career out of just following Judah Folkman around and saying, "Gee whiz!" And I would like to sign up for the job, because just when I think I know something, he sort of takes it, turns it upside down, and then says, "Okay, Smarty, now what?"

And I was just thinking, "Now what?" The whole idea of the role of acid in ulcerogenesis has taken a lot of body blows in the last 2 years. We should never forget that Dick Varco and Owen Wangensteen took rats and injected histamine in beeswax, and that is all they did. And every one of those rats perforated a duodenal ulcer and died. And all the histamine in the beeswax did as far as we know was to stimulate acid secretion.

About 20 years ago, Mort Grossman reported that acid secretion in man diminishes with age. We had a young man, Talaet Khalil, from Egypt in our laboratory at that time, and he took some rats at different ages and exactly duplicated the data from man in rats.

Just about that time, a man named John Kurata at CURE did an analysis of rates of duodenal ulcer appearance in man all over the world with aging and found that the older you get, the more duodenal ulcers you get. Well that posed a kind of paradox. Your acid production goes down with aging—that is easily demonstrated—and duodenal ulcer production goes up.

Along about that time, Jon Isenberg made the observation that many patients with duodenal ulcer disease have defective mechanisms for production of duodenal bicarbonate.

We had a young man from Korea named Kim, and Dr. Kim took rats of different ages, measured their duodenal bicarbonate production, and found that they did not have any duodenal bicarbonate production when they got old. It went away. He then found that if you gave them vasoactive intestinal polypeptide (VIP) or prostaglandins, which are the two mechanisms for stimulation of duodenal bicarbonate production, duodenal bicarbonate production was okay.

So it was an age-related defect in the sensory mechanism, the mechanism to detect acid, or to stimulate the signal from acid detection to release VIP or prostaglandin, that was responsible.

So we know that there are at least those two mechanisms involved in ulcerogenesis; that is acid clearly causes them; some problem having to do with production of endogenous bicarbonate clearly causes them. There is a whole camp of people out there on the periphery reminding us about helicobacter pylori and about its role in the genesis of ulcer, which I do not fully understand.

I think that Dr. Folkman and his colleagues have probably come upon a very central, basic mechanism that goes beyond all of these, I suspect, fairly ancillary observations.

I would like to ask Dr. Folkman, does he by any chance have any data on the relationship of classic defense mechanisms, such as mucosal bicarbonate production, and this fibroblast growth factor, or its effect on acid production?

DR. MARSHALL J. ORLOFF (San Diego, California): I guess it is true of all of us. I am regularly awed by Judah's thoughts about global matters, and about the methodology and rapidity with which he investigates those thoughts, even if not all of these prove to be true.

I wanted to ask one question, Judah, and it is regarding the histology of the sucralfate-treated ulcers. You showed us those beautiful slides of angiogenesis in the ulcers that had been treated by FGF. If sucralfate acts in the same way in preserving endogenous FGF, I would expect that one would see the same kind of angiogenesis in those treated ulcers. Did you look at those ulcers and examine them in the same way?

The second comment I have is that it would be interesting to know whether the amount of fibrogenesis and collagen produced in the FGF-treated ulcers was different from that in ulcers treated by conventional means. Is a greater scar produced? Is there more collagen? Is there more fibrogenesis? If so FGF might have some potential disadvantages.

Wonderful study.

DR. PAUL S. RUSSELL (Boston, Massachusetts): I want to ask one question. I think Bill Silen referred to this previously. As I understand it, Judah is presenting us with a hypothesis as to the healing or nonhealing of existing duodenal ulcers. A question that remains is whether or not the ulcers come to pass in the first place as a consequence of some aberration in angiogenic factors?

Were that to be true, it might be that the integrity of all our tissues is sponsored by and dependent on the presence of angiogenic factors constantly being present. Is that part of your hypothesis?

DR. WARD O. GRIFFEN, JR (Philadelphia, Pennsylvania): Judah, while you are coming up to close, I will just ask a question, too. You showed that there was FGF in the human duodenum. Is that a normal human duodenum, or is that the duodenum of a patient who has a duodenal ulcer, or do you know whether FGF is present in the duodena of patients with ulcers?

DR. JUDAH FOLKMAN (Closing discussion): I would like to thank the discussants for their comments.

Dr. Griffen, the duodenal mucosa was from a patient who actually had a fistula from colon to stomach, and that duodenum was not ulcerated.

Dr. Silen's points are well taken. Can one separate contraction from angiogenesis? The only evidence we have for the role of these two together is that when we use angiogenesis inhibitors on wounds in mice, the wound contracture is delayed. That is indirect evidence, however. My personal feeling is that wound contraction is angiogenesis dependent. But I feel that about everything; my income is angiogenesis dependent.

There are other growth factors involved in the healing of peptic ulcers. Epidermal growth factor is important. It also lowers acid. Our data with FGF show more rapid healing of duodenal ulcers than the reports on EGF. We have tested FGF on other kinds of ulceration, for example colonic ulceration produced by acetic acid. That is the work of H. Satoh and Sandor Szabo.

I appreciate Jim Thompson's remarks. The relationship of helicobacter pylori to peptic ulceration is an interesting one. We would certainly like to know whether this bacteria interferes with production of FGF in the stomach.

In answer to the question about the effect on bicarbonate, we have not looked at that.

Marshall Orloff asked about the histology of the sucralfate-treated ulcers. These sections are still pending, but we have done extensive work with sucralfate in other tissues. Sucralfate is angiogenic in the rabbit cornea, in the chick embryo, and in the rat subcutaneous pouch. By itself it is not inflammatory. In the cornea it appears to extract FGF and then slowly dissolve and release it. If you add FGF to the sucralfate before implantation, one sees intense angiogenesis.

There does not seem to be increased fibrogenesis in the treated ulcer bed, but we have not measured collagen levels.

Paul Russell asked a very interesting question about the difference between the role of FGF in prevention of ulcers *versus* therapy of ulcers. Here we were trying to see if we could explain the mechanism of all common anti-ulcer therapy by a single hypothesis. We propose that they all operate by protecting endogenous FGF from being inactivated by acid. By endogenous FGF I mean FGF produced in the ulcer bed as well as by the gastric and duodenal mucosa.

This leads to the question that, if you were able to deplete endogenous FGF, could you delay ulcer healing? Mark Stovroff has some interesting preliminary data that support that idea. He and Yuen Shing in our laboratory fed sucrose octasulfate (the soluble form of sucralfate) to rats with duodenal ulcers, and the level of endogenous FGF was significantly decreased. Dr. Vattay, in Sandor Szabo's laboratory at the Brigham and Women's Hospital, has further preliminary evidence that feeding sucrose octasulfate will retard healing of duodenal ulcers. We think this is because the sucrose octasulfate continuously extracts FGF from the stomach lining.

Thank you very much.

Liver Transplantation in Children From Living Related Donors

Surgical Techniques and Results

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Pediatric liver transplantation with reduced size donor organs (RLT) has evolved into a standard clinical procedure increasing the choices of recipients for their treatment. Nevertheless organ availability remains a major problem. The authors therefore have proposed to study the use of hepatic segments from living related donors (LRT) in a group of 20 children less than 2 years of age or weighing less than 15 kg, in whom standard indications for transplantation existed. Volunteer related donors were selected after medical and psychiatric evaluations, and the suitability of the donor's liver was established by functional and radiologic criteria. A two-stage informed consent process assured appropriate "volunteerism." Nineteen infants received LRT as first grafts and one as a second graft. Seventeen of the recipients are alive 3 to 18 months after LRT. Fifteen of 20 patients are currently at home with the original graft and normal liver function (bilirubin < 1.5 mg/dl) after a median hospital stay of 27 days (range, 14–93 days). Four patients underwent retransplantation, in all cases due to arterial thrombosis. The overall graft survival for 20 primary LRTs is 75%, with follow-up between 3 and 18 months. A number of technical problems occurred during our initial trial, the most aggravating being vascular thrombosis. Refined approaches to vascular reconstruction should reduce the incidence of thrombosis and improve the rate of survival in future cases. The donor group for the initial 20 LRT procedures comprised 12 mothers, 7 fathers, and 1 grandmother. In addition one father and one uncle, who was an identical twin of the recipient's father, who did not qualify for anatomic reasons, were used in repeat LRT. All donors survived and are now in normal health between 3 and 18 months after LRT, having returned to all activities enjoyed before donation. The median hospital stay was 6 days (range, 5–14). Complications were minimal, and all were limited to the first three procedures, in which a full left hepatectomy was performed. After alteration of the procedure into a left lateral segmentectomy, no complications were encountered. The left lateral segmentectomy presents minimal surgical trauma to the liver and should remain the primary approach for obtaining a liver graft from a living donor. For children, transplantation of a left lateral segment from a live donor provides a new way of providing a transplant of appropriate size and with good function. The success of this program has led to the acceptance of LRT for general clinical application in the authors' institution.

THE CLINICAL APPLICATION of orthotopic liver transplantation (OLT) using living donors (LRT) has not emerged until recently because of ethical considerations[1] and perceived technical difficulties. The precursors of LRT have gained acceptance in the transplantation community, setting the stage for the use of living donors. Reduced-size liver transplantation (RLT) was initially introduced in Europe in 1985[2,3] and first used in North American by our group.[4] In 1987 we documented using equipoise analysis that the routine use of RLT provides a significant survival advantage in pediatric liver transplantation because of the epidemiology of liver disease in infants *versus* that of organ donors—more infants die of liver disease than become organ donors.[5] In most major pediatric centers, RLT has become routine in the treatment of small pediatric recipients.[6–10] Within the last 2 years, 78% of all pediatric transplants performed in our center have been performed with reduced-size donor organs. Several centers have reported success rates with RLT in the range of 70% to 80% 1-year patient survival, which is comparable to the success with full-size

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transplants in small recipients. Indeed the incidence of arterial thrombosis has been reported to be lower when segments from adult donors have been used as compared with small donor organs.[6] The complete success of RLT provides the surgical foundation for using segments from living donors for the treatment of small children.

Since 1985 there has been a linear increase in the number of liver transplants performed in the United States that has outpaced the availability of organ donors, which has shown no growth. There is a considerable risk at present for a candidate to die on the waiting list before OLT[11]; the risk may be greater for adults than for children because of the more rapidly expanding indications and demand for OLT in adults. These changes are producing pressures to limit the use of RLT, which are expected to mount over the next few years.

The progressive shortage of donors for pediatric OLT has prompted our group to explore alternative approaches; we first explored the use of split-liver OLT (SLT), where one donor provides grafts for two recipients,[12] and we have re-explored the use of LRT.[1] The primary intermediate advance between RLT and LRT was the performance of SLT, initially performed by Pichlmayr et al.,[13] because using one liver to treat two recipients demonstrated the feasibility of reconstructing the vascular and biliary pedicle of the left lateral lobe graft for implantation into a small recipient.[12] The increasing success with reduced size livers and split liver segments provided the basis for the use of segments from live donors.

The remaining obstacle that had to be overcome before a program of LRT could be initiated was the ethical dilemma of subjecting a healthy person to major hepatic resection to obtain a graft for a child with liver disease. We undertook an exhaustive analysis of the ethical issues concerning LRT[1] and concluded that the surgical risk was low enough to be acceptable for a parent or other close relative if they volunteered to be an organ donor. Surgical risks to the donor were estimated from observed rates of complications in patients undergoing left lobectomy in our institution. Psychological risks and benefits could not be estimated and were to be a focus of study in our first series of LRTs.

The clinical use of LRT therefore has been made possible by recent advances in hepatic surgery: first improved understanding of the anatomy and techniques of hepatic resection[14]; second growing evidence that the operative risk of partial hepatectomy in a noncirrhotic patient is extremely low[15,16]; third widespread success with RLT[6-10]; and fourth the successful application of SLT.[12] Clinical ethical consultation was also an important precursor to the first LRT.[1] Based on these foundations, we undertook a trial of LRT in a series of 20 infants. We report herein the surgical techniques and results in these patients.

Patients and Methods

Selection of Donors and Recipients

The criteria for selection of families for participation in the LRT program have been published.[1] Briefly, recipients were limited to infants with body weight less than 15 kg and with an accepted indication for OLT.[17] We excluded patients with emergency indications and those whose medical condition was judged to not be stable enough to permit donor evaluation and informed consent to be obtained, which required 2 weeks. Because of funding considerations, all but two families carried health insurance that covered the costs of evaluation and LRT; two were funded by state aid.

Donors were relatives (parents in all but one case) who volunteered for participation; no families were solicited, but rather were considered if they requested involvement. The donor informed consent process has been detailed elsewhere. It involved a full description of potential complications and described in detail all complications that had been observed in preceding cases. Every effort was made to reduce the pressure to volunteer by emphasizing the effectiveness of cadaver grafting and the high likelihood (approximately 98% in our series) of obtaining a liver because of the availability of RLT. If, after discussion, both parents volunteered as donors and both were generally compatible (blood type, size, etc.), they were asked to decide who was to be evaluated. We excluded single parents. In one case both parents were judged to be incompatible (one because of blood type and one because of size), and a grandmother was accepted for evaluation. Donors underwent thorough medical and psychiatric examination to determine their fitness for undergoing major surgery. ABO blood group compatibility was required. A computed tomography (CT) scan of the liver was performed and an estimate of the size of the left lateral lobe (segments 2 and 3) was obtained using an established technique of three-dimensional computerized imaging.[18] The estimated volume of the left lateral segment was compared with the size of the recipient, and some potential donors were excluded because of size incompatibility. In general grafts with a predicted volume of less than 350 ml were accepted. Selective celiac and mesenteric angiography was performed for identification of the vascular anatomy when all other evaluations were completed.

Surgical Procedures

Donor Procedure. The donor hepatectomy was performed through a bilateral subcostal incision with median extension to the xiphoid process (Fig. 1). In all cases autologous blood was returned through a cell saver system.

An anatomic left hepatectomy was performed in the first three cases. The resection included mobilization of

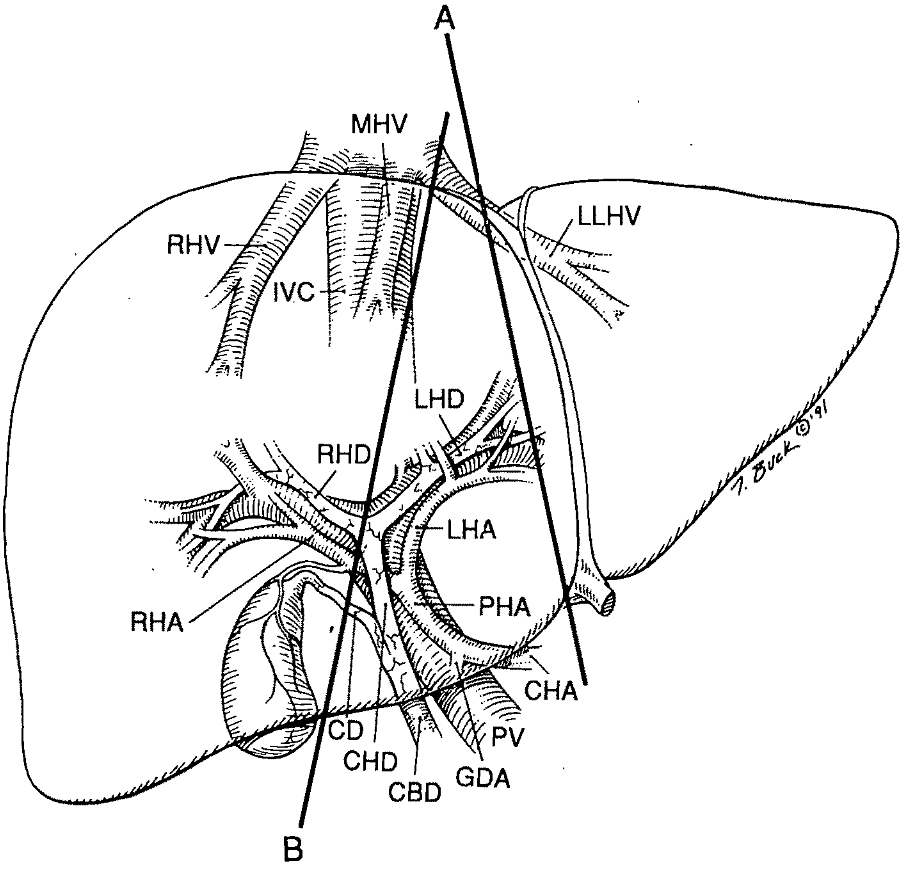


FIG. 1. Overall depiction of the donor operation. The relationships between the portal and hepatic venous elements are illustrated. Planes of parenchymal transection are presented for the creation of a segment 2 and 3 graft (A), and a full left lobe graft (B). LLHV, left hepatic vein, MHV, middle hepatic vein, IVC, inferior vena cava, RHV, right hepatic vein, RHA, right hepatic artery, RHD, right hepatic duct, LHD, left hepatic duct, LHA, left hepatic artery, PHA, proper hepatic artery, CD, cystic duct, CHD, common hepatic duct, CBD, common bile duct, GDA, gastroduodenal artery, CHA, common hepatic artery, PV, portal vein.

segments 1 through 4 and removal of the gallbladder. Parenchymal transection was done during vascular exclusion after isolation of the hepatoduodenal ligament, the hepatic veins, and the infrahepatic vena cava. During resection of the lobe, suture ligations were placed only on the donor resection plane, and the liver was recirculated immediately on completion of the resection. The warm ischemia time of the donor liver was less than 25 minutes in each case. After removal, perfusion of the left lobe with heparinized University of Wisconsin solution (Viaspan, Dupont, Wilmington, Delaware) through the portal vein and the hepatic artery was performed on a separate table. During cold ischemia, segment 4 was resected from the graft, thus preserving sufficient length of the vascular pedicles and the bile duct. The parenchyma was divided at the falciform ligament and the vascular structures suture ligated. The left hepatic vein was preserved at its junction with the median left hepatic vein.

After the third case, the donor resection was limited to resection of segments 2 and 3. In this procedure the initial maneuver was to isolate the left hepatic artery, either branching off the common hepatic artery or coming from the left gastric artery. The hilum was entered to the right of the round ligament, disconnecting portal branches to segment 4, but preserving arterial and biliary elements when possible. The left portal vein was encircled, and the branches to the caudate lobe were divided to assure adequate mobility. Following the round ligament posteriorly between segments 2, 3, and segment 1, the bile duct was isolated and severed.

The hepatic vein of segments 2 and 3 was encircled, and without interruption of local circulation, the hepatic parenchyma was dissected by mosquito fraction technique until the left lateral segment was completely separated from the median left segment. Suture ligations were placed on both the donor and the graft resection planes. Simultaneous clamping of the donor's vascular pedicles allowed removal of the lateral segment without warm ischemia, and immediate flushing with cold preservation solution was performed *ex situ.*

Figures 2 and 3 demonstrate the varying biliary and arterial dissections that were required to create adequate pedicles for anastomosis. In seen cases the bile duct from segments 2 and 3 did not form a common trunk before entry of the duct from segment 4. The anterior branch (segment 3) was usually in close proximity to the artery, whereas the posterior branch (segment 2) followed the portal vein.

The arterial anatomy presented several variants that required modification of the dissection. In patients with

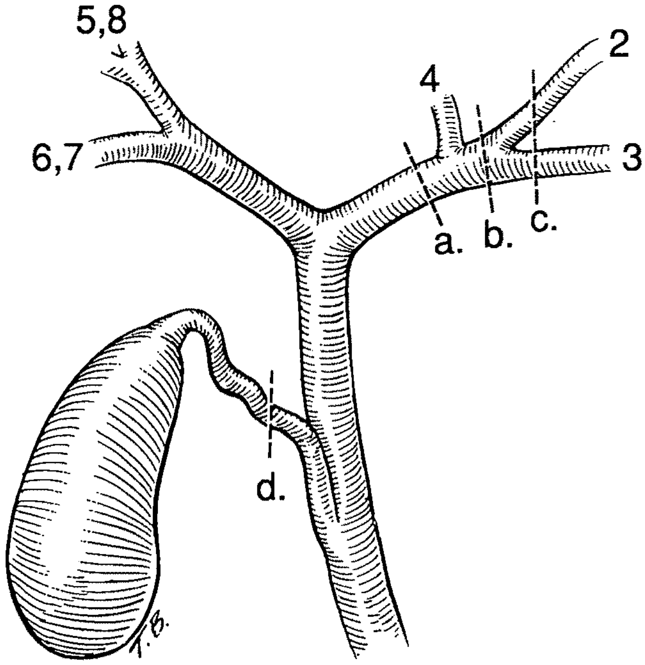


FIG. 2. Normal biliary tract anatomy. Section (A) divides the main left duct. When possible section (B) results in a single duct for a segment 2 and 3 graft. A more distal division (C) results in two separate orifices for reconstruction. Cholecystectomy (D) is not required for the biliary dissection.

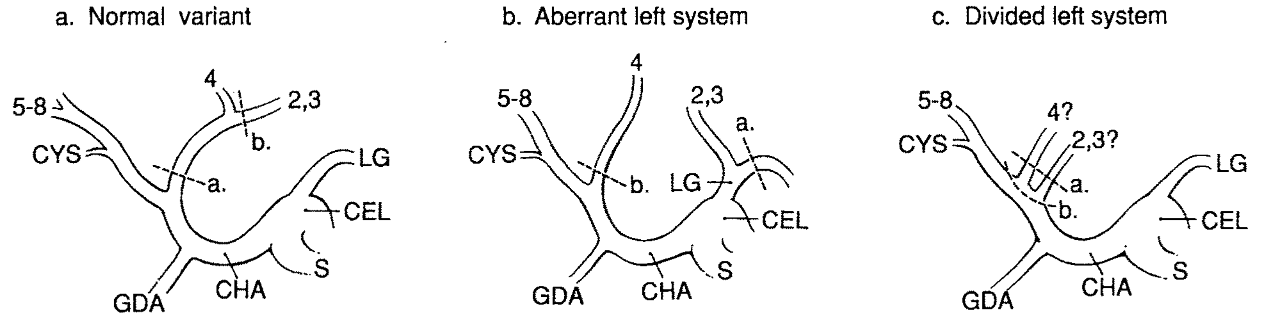
normal anatomy (Fig. 3A), a single branch was prepared, when possible, without interrupting the supply to segment 4. As a result the artery was often small and short. In one case, the anterior portion of segment 4 had to be removed through a wedge resection because of avascular demarcation. In patients with a left hepatic artery originating from the left gastric artery (Fig. 3B), the artery to segment 4 could always be preserved. Patients with a divided left system were the most difficult (Fig. 3C) and required reconstruction of two smaller vessels.

Recipient Operation. The technique for implantation of the segment 2, 3 graft is a modification of that used in split liver transplantation for the left-sided graft (Fig. 4), which has been elaborated in detail in a separate publication.[12] Although several modifications were attempted in this series, it appears that the essential elements include: first rotation of the graft to the right with a wide venous anastomosis; second extension of the hepatic artery and portal vein with interposition grafts; third direct use of the aorta for arterial inflow. Care must be taken to correctly identify the ducts for segments 2 and 3. In this series separate ducts were encountered in 7 of 17 cases (41%) in which the transection was made at the round ligament.

In the first three cases, using the entire left lobe, the arteries were larger and the portal vein was of sufficient length. In cases in which segments 2 and 3 were used, both vascular pedicles required reconstruction. In two cases, a double left hepatic artery was reconstructed into a single lumen. In three cases the artery was extended by means of an inferior mesenteric vein interposition. Although this procedure avoided a separate incision in the groin of the donor for extraction of the saphenous vein, a saphenous vein segment was obtained in subsequent cases, as it provided a more satisfactory arterial substitute than the thin-walled mesenteric vein, which dilated and thrombosed in two cases.

Postoperative Care

Standard post-OLT care was provided by the combined surgical and pediatric liver service. Immunosuppression was based on our standard regimen, using cyclosporine, azathioprine, and steroids.[19] Cyclosporine was administered intravenously in a dosage of 2 mg/kg/day, divided every 12 hours, from the first postoperative day, which was increased to 4 mg/kg/day on postoperative day 3. From postoperative day 5, intravenous or oral dosing was


a. Normal variant

b. Aberrant left system

c. Divided left system

FIG. 3. Schematic depiction of hepatic arterial anatomy relevant to LRT. (A) Normal variant with a single artery for segments 2, 3, and 4. Transection can be made at the origin, or more distally (b) to preserve the segment 4 vessel. (B) Left hepatic artery originating from the left gastric usually supplies segments 2 and 3. Transection at point (a) can produce a long vessel for anastomosis. The branch to segment 4 can be transected (b) or preserved with the donor. (C) Two separate arteries of equal size can supply the left liver. This variant is the least favorable, transection (a) results in two small vessels which need to be joined, (b) can potentially compromise the donor right artery. CEL, celiac artery, S, splenic artery, CHA, common hepatic artery, GDA, gastroduodenal artery, CYS, cystic artery, LG, left gastric artery.

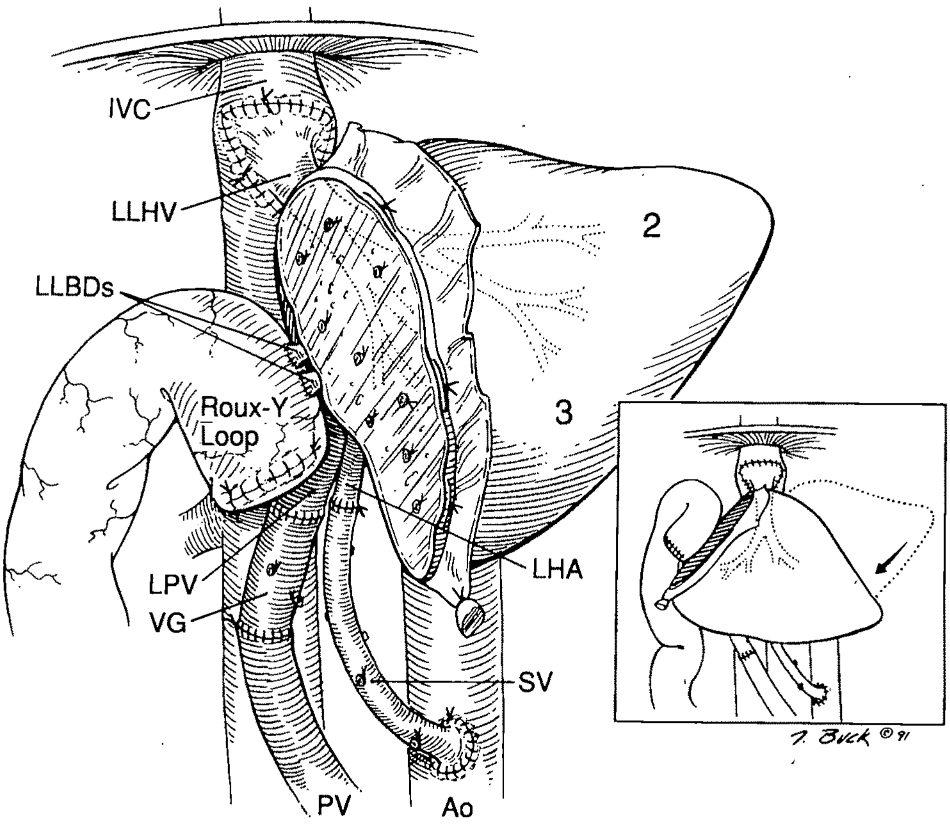


FIG. 4. Completed recipient implantation. Variant with two bile ducts is depicted. Interposition grafts are used for both hepatic artery and portal vein to assure adequate length. *Insert* shows final position of the graft after abdominal closure. LLBDs, segment 2 and 3 bile ducts, LPV, left portal vein, VG, vein graft, SV, saphenous vein, Ao, aorta.

adjusted to achieve a 12-hour trough level of 200 ng/ml of whole blood as determined by high-performance liquid chromatography.[20] Azathioprine was administered at a constant dosage of 1 mg/kg/day. Methylprednisolone was administered intravenously every 12 hours at a dosage of 2 mg/kg/day on postoperative day 1, which was linearly tapered to 0.3 mg/kg/day by postoperative day 7. Rejection was diagnosed by clinical criteria and always confirmed by histology. Rejection management followed published protocol; initial therapy involved pulse corticosteroid therapy (10 mg methylprednisolone/kg/day for 3 days), whereas resistant rejection was treated with OKT3 (2 cases) and FK506 (1 case).

Results

Recipients

The demographic and medical status of recipients is summarized in Table 1. Twenty infants were treated between November 27, 1989 and February 9, 1991. Recipients were 15.5 ± 11.5 months old at the time of LRT, and weighed 8.4 ± 2.5 kg. The indications for transplantation were biliary atresia in 16 cases, a_1-antitrypsin deficiency in 2, cytomegalovirus (CMV) hepatitis in 1, and neonatal hepatitis in 1. One patient with a_1-antitrypsin deficiency had received a cadaver graft and had suffered arterial thrombosis 3 weeks before LRT, which was performed for the indication of persistent hepatic infection. Living related donor graft was performed in 14 children who required constant medical therapy, but had no major complications. These patients would fulfill current United Network for Organ Sharing (UNOS) criteria for listing for a cadaver graft at status 1, and LRT was therefore considered to be an elective procedure. In the remaining

TABLE 1. *Demographic and Clinical Description of Recipients*

Characteristic	No.
Sex	
M	6
F	14
Age (m)	15.5 ± 11.5*
Weight (kg)	8.5 ± 2.5
Diagnosis	
Biliary atresia	16 (80%)
a_1-antitrypsin deficiency	2 (10%)
CMV hepatitis	1 (5%)
Giant cell hepatitis	1 (5%)
UNOS status†	
1	14 (70%)
2	1 (10%)
3	4 (20%)
4	1 (5%)

* Mean ± SD.

† National organ sharing classification: 1, at home; 2, requiring constant care; 3, in the hospital; 4, critically ill, requiring intensive care.

UNOS, United Network for Organ Sharing.

six cases, the recipients had suffered intermittent episodes of severe complications, but were medically stable and met protocol criteria at the time of LRT. One of these cases would meet UNOS criteria for listing at status 2, four at status 3, and one at status 4. This last patient decompensated after the donor evaluation had been completed and final consent was obtained; the case was judged to meet protocol criteria on this basis.

Donors

Table 2 summarizes the donors and graft sizes obtained in 20 cases. The ages of the donors ranged from 21 to 41 years. Twelve donors were mothers of recipients, seven were fathers, and one was a grandmother. The size of the left lateral segment as assessed by CT scanning averaged 250 ml. Arteriography demonstrated a high incidence of anomalies of the hepatic arterial circulation (Table 3). Initially the existence of a double arterial supply for the left lobe was considered acceptable, but subsequently, because of technical difficulties with reconstruction, only anatomic variants with a single arterial supply for the potential graft were used. Two potential donors were rejected for size discrepancy and three for reasons related to the hepatic arterial anatomy.

Donor Survival and Complications

Results of surgical procedures in 20 donors are summarized in Table 4. All donors survived and are now in normal health between 3 and 18 months after LRT, having returned to all activities enjoyed before donation. The median hospital stay was 6 days (range, 5–14). The median blood loss during the operation was 350 mL. The first donor required 4 units of red cell replacement (2 were previously donated autologous units); since then no other donor has required heterologous transfusion. Complications occurred only in the first three cases. During the first case, the spleen ruptured while vascular exclusion was applied, probably because of the sudden increase in splanchnic pressure combined with retractor trauma. This same patient had a bile leak from the cut surface that required percutaneous drainage. One other donor had a bile leak from the cut surface that required prolonged percutaneous drainage before drying up, and the other had a subphrenic fluid collection that required a one-time percutaneous evacuation. In the remaining 17 donors, no abdominal problems occurred. Lymphoceles developed in the groins of two patients after removal of the saphenous vein.

TABLE 2. *Description of Donors and Graft Volume Obtained*

Description	No.
Demographic	
Relation to recipient	
Mother	12
Father	7
Grandmother	1
Age (yr)	26 (20–42)*
Weight (kg)	66.1 (50–92)†
Graft	
Graft volume (ml)	250.2 ± 65.3‡
Graft volume/kg recipient	32.3 ± 8.3‡

* Median (range).
† Mean (range).
‡ Mean ± SD.

TABLE 3. *Variants of Arterial Anatomy in 20 Donors Assessed by Arteriography*

Anatomic Variant	No.*
Normal	10 (50%)
L-hepatic from L-gastric	5 (25%)
R-hepatic from SMA	3 (15%)
2 L-hepatic arteries	3 (15%)

* One patient had two variants.

Two donors (a father and the identical twin of a father) provided grafts for retransplantation of two children and are not included in the initial series; they had uneventful postoperative courses and are currently well.

Recipient and Graft Survival

Nineteen recipients received LRT as primary grafts, and one as a second graft. Seventeen of the recipients are alive 3 to 18 months after LRT (actual survival is 85%, actuarial 1-year survival is 82%) (Table 5). One child with a functioning primary graft died from pneumococcal sepsis 5 months after LRT during a lapse in penicillin prophylaxis. After the transplant procedure, the child's massive spleen had to be removed because of mechanical ventilation problems. The patient had chronic rejection requiring therapy with FK-506, but at the time of death, the liver function was nearly normal.

Fifteen of twenty patients are currently at home with the original graft and normal liver function (bilirubin <1.5 mg/dL) after a median hospital stay of 25 days (range, 14–68).

TABLE 4. *Postoperative Complications and Survival in 20 Donors*

Observation	No.
Alive and well	20 (100%)
Complications	
Splenectomy	1 (5%)
Fluid collection	1 (5%)
Lymphocele	2 (10%)
Bile leak	2 (10%)
Reoperation	1 (5%)
Hospital stay (days)	6 (5–14)*

* Median (range).

TABLE 5. *Patient and Graft Outcome in Recipients of LRT (Follow-up 3 to 18 months)*

Status	No.
Patient	
Alive with primary graft	15 (75%)
Alive with retransplant	2 (10%)
Currently hospitalized	1 (5%)
Total actual patient survival	17 (85%)
Graft	
Intact primary graft	15 (75%)
Lost to arterial thrombosis	4 (20%)
Lost to patient death	1 (5%)
Total actual graft survival	15 (75%)

LRT, living-related transplantation.

TABLE 6. *Variants of Arterial Implantat in Recipients of LRT*

Technique	No. (%)	Thrombosis
To hepatic artery	8 (40%)	4 (50%)
End to end	3 (15%)	1 (33%)
With interposition	5 (25%)	3 (60%)
To aorta	12 (60%)	1 (8%)*
Total		5 (25%)

* Small graft, dual left arteries.
LRT, living-related transplantation.

Retransplantation

Four patients underwent retransplantation, in all cases due to arterial thrombosis. The overall graft survival for 20 primary LRTs is 75% with follow-up between 3 and 18 months. Two patients are alive with retransplants, one with LRT from another relative, and one with a cadaver graft.

Retransplantation was due to arterial thrombosis in all cases, which appears to be the major difficulty with LRT. Two patients developed arterial thrombosis in the early postoperative period and received cadaveric grafts as emergency procedures. The first developed arterial thrombosis intraoperatively because of the use of a very small saphenous vein. Although thrombectomy was initially successful, clot had apparently propagated into the distal arterial tree and thrombosis recurred within 24 hours and resulted in necrosis of the bile duct. On day 4, she was retransplanted with a cadaveric RLT, but polymicrobial intra-abdominal infection and mycotic disruption of the aortic anastomosis resulted in her death 12 days after retransplantation. The second patient developed arterial and portal vein thrombosis with graft necrosis within 12 hours after LRT, apparently the result of swelling and change in the position of the graft. He was regrafted with a size-matched liver within 48 hours, but arterial thrombosis reoccurred on postoperative day 4, causing breakdown of the biliary anastomosis. He received a third graft from his uncle, an identical twin of his father, who was excluded because of arterial anatomy. This patient died after 3 months as a result of complications of CMV infection. Two patients developed thrombosis at least 14 days after LRT. One received a cadaveric RLT 4 weeks after LRT when arterial thrombosis led to partial infarction of the graft. Because liver function was normal, attempts were made to salvage the graft by removing the infarcted portion, but a biliary fistula developed. Recurrent infections and inability to maintain immunosuppression mandated graft replacement. This patient has subsequently developed severe rejection, which has required long-term management with FK506, and he has been plagued with opportunistic infections during the 12-month period since replacement of the primary graft. The other patient was retransplanted for arterial thrombosis diagnosed on day 15. Initially liver function remained normal until bacterial and fungal sepsis associated with bile duct breakdown occurred. Because both parents were suitable donors, the father was able to provide the retransplant. The child is currently hospitalized for nutritional repletion and has improving liver function.

It should be noted that no graft was lost because of primary nonfunction.

Vascular Complications

Table 6 summarizes the types of arterial anastomosis and the incidence of thrombosis. Thrombosis was common when the recipient hepatic artery was used for inflow. The only thrombosis that occurred with the use of the aortic inflow was the result of a small-sized saphenous vein. In three cases two small arteries supplied the graft; this necessitated reconstruction into a single lumen (Fig. 3c). Thrombosis occurred in two of these cases. In one primary graft and one retransplant, arterial thrombosis was successfully managed with thrombectomy and reconstruction using a cryopreserved saphenous vein.

Portal venous reconstructions (Table 7) were performed initially without an interposition graft. The high incidence of thrombosis led us to systematically extend the vein to avoid tension. Portal veins were extended using either donor inferior mesenteric vein or saphenous vein (both of which required extensive suturing to double the circumference), or iliac vein from a cadaver donor. A total of four portal vein thromboses occurred intraoperatively and postoperatively; all were managed successfully by

TABLE 7. *Variants of Portal Vein Reconstruction: Relationship Between Method and Incidence of Thrombosis*

Technique	No.	Thrombosis
Primary anastomosis	14 (70%)	4 (29%)
Interposition	6 (30%)	0
Total	20 (100%)	4 (20%)

thrombectomy and reconstruction. Portal vein thrombosis has not occurred when a long interposition has been placed to prevent tension.

Biliary Complications

Overall biliary complications occurred in 7 of 20 cases (35%). In all five cases of arterial thrombosis, breakdown of the biliary anastomosis occurred before retransplantation. In one case a posterior bile duct of the lateral segment was overlooked and required drainage 5 weeks after transplant. The diagnosis was made by ultrasound and monitoring of cholestatic enzymes. Functionally the transplant was normal. In two cases a small leak required laparotomy and stenting within the first 2 postoperative weeks. Seven months after transplantation, a stricture was repaired in another patient.

Rejection Episodes

In 17 primary grafts functioning for more than 3 weeks 8 rejection episodes occurred (Table 8), as evidenced by clinical assessment and with biopsy confirmation. Two patients had mild rejection requiring no specific treatment. Three responded to corticosteroid pulse therapy and have suffered no second rejection episode. Two children required treatment with OKT3 for steroid-resistant rejection. One patient subsequently required FK506 for continued control of rejection. The overall incidence of rejection requiring treatment was 35% in LRT, and there was no graft loss of a primary LRT from rejection.

Discussion

The procedure of using living related donors for liver transplantation was developed at our institution in several steps. The successful use of RLT and SLT provided the basis for the use of segments from living donors and the necessary surgical prerequisite to virtually assure success of LRT from the perspective of the recipient. Overcoming the ethical problems, which required intensive study and reflection, was the key hurdle before a program could be implemented. While addressing these problems and before our program was active, four live donor operations were performed elsewhere, two in Brazil,[21] one in Australia,[22] and one in Japan. In those countries pediatric donor organs are extremely scarce and the procedures were performed as an individual approach to salvage the lives of infants with no other chance of treatment. Although these were pioneering efforts, our goal was to study LRT in the context of an active transplant program with established expertise in RLT and SLT. In this way the relative advantages or disadvantages of LRT could be assessed. The research protocol called for a discrete cohort of infants to be studied before LRT would be accepted, or not, for general clinical application. Living related donor transplant was to be evaluated as a surgical approach to respond to increased needs in the future.

TABLE 8. *Characterization of Rejection Episodes in LRT Observed More than 3 Weeks (N = 17)*

Severity	No.
No therapy required	2 (12%)
Steroid responsive	3 (17%)
Steroid resistant	3 (17%)
OKT3	2 (12%)
FK506	1 (6%)
Graft loss to rejection	0 (0%)
Total incidence of rejection	8 (47%)

LRT, living-related transplantation.

The surgical anatomy of the liver allows for lobar or segmental resection in the treatment of hepatic tumors.[14] In patients with normal liver function and no general medical risk factors, hepatic resections have been performed with little or no deaths.[15,16] Consequently we assumed that, with appropriate experience in hepatic resections, the donor in an LRT procedure would be placed at low risk. The risk to the donor was our crucial concern, and therefore we prepared to perform the least traumatizing hepatectomy possible, removing the amount of liver tissue needed to prepare an adequate graft. Initially left hepatectomy seemed to fulfill those criteria and therefore was performed in a standard fashion with vascular exclusion technique. The removal of the entire lobe would, we anticipated, eliminate most problems presented by anomalies of the arterial and biliary systems. The left lobe was to be reduced *ex vivo* into a left lateral segment, thereby preserving sufficient length of the hepatic artery and the portal vein.

After the first three procedures were completed, each with complications, the procedure was limited to a left lateral segmentectomy. It was clear from our experience that the vascular pedicles would be smaller and shorter and would require extensive reconstruction. Moreover the bile ducts would be considerably smaller and complications could arise from a potential compromise of the circulation of the median left lobe. The surgical trauma to the donor, the loss of liver tissue that could be spared, and the likelihood of traumatizing vital hilar structures with left hepatectomy seemed to favor a more limited procedure, however. We decided that the safety of the donor would take absolute priority over potential minor impairment of the graft quality. With this compromise segments obtained from live donors would not be comparable to RLT from cadaveric donors, which contain the entire common hepatic artery, a full length of portal vein, and a single bile duct, but would be more like left liver grafts for SLT. As the success rates for SLT are not equivalent to those for RLT, the recipient would be placed at some risk, which deserved study.

The removal of the left lateral segment, as we perform the procedure, presents the smallest possible trauma to the donor's liver. Only approximately 25% of the liver mass is removed, which has had no effect on routine liver function tests in our experience. Vital hilar structures on the remaining right side are virtually untouched and the ligamenta on the right side remain intact. This makes the operation much easier and reduces the trauma to the donor, and potential complications resulting from full mobilization of the liver. Potential problems at the site of the hepatic venous junction are easier to control, because the vena cava and the caudate lobe are not traumatized through dissection, as would be required for a left hepatectomy. The confluence of the bile ducts does not require dissection, cholecystectomy need not be performed, and a T-tube cholangiostomy is not necessary, preventing traumatization and future scarring.

The recipient operation did not differ from routine cadaveric transplants in terms of blood loss, cross-clamping time, and intraoperative requirements. In contrast to cadaver graft transplant procedures, however, within an 8-hour period coagulation parameters were corrected to normal, and the reoperation rate for bleeding was sharply reduced. The circulation of the grafts was homogenous from the very beginning and the grafts were extremely soft as compared with cadaver grafts. They were, as a result, easy to traumatize. In the first case, the recipient developed subcapsular bleeding from minor trauma, which developed into major hemorrhage and resulted in an episode of shock. Two other recipients experienced significant bleeding from percutaneous liver biopsy for the diagnosis of graft dysfunction, which has not been observed in over 500 biopsies performed in recipients of cadaver segments. This probably represents an indication of the excellent quality of the graft from a living donor, but is a surgical problem to be considered.

A number of technical problems occurred during our initial trial, the most aggravating being arterial thrombosis. Five patients developed arterial thrombosis, a rate that is somewhat higher than our usual experience. In the initial more limited left lateral segmentectomy cases needing extension of the hepatic artery, the vascular reconstructions were performed with an inferior mesenteric vein graft to avoid an inguinal incision for extraction of a piece of the saphenous vein. The use of the inferior mesenteric vein turned out to be inappropriate for the arterial reconstruction, even when a small length was used. The veins became ectatic when exposed to arterial pressure, which caused turbulence, and thrombosis resulted. Subsequently a saphenous vein graft was routinely obtained for arterial reconstruction. Regardless of the length of the interposition of the saphenous vein, anastomosis to the recipient's common hepatic artery resulted in a high incidence of thrombosis, whereas anastomosis to the infrarenal aorta nearly always remained patent. At present therefore we are routinely attaching the hepatic artery through a saphenous interposition to the aorta. We believe this represents the optimal approach, against which other novel approaches must be compared. We now avoid using donors with two arteries supplying the left liver because of the very high risk for thrombosis, occurring in two of three patients in whom it was attempted (Fig. 3c). This represents one of the main reasons to continue the practice of preoperative arteriograms in all donors.

Sufficient length of the portal vein has been required to appropriately position the graft with a slight rotation to the right side, with the cut surface facing the retroperitoneum (Fig. 4). The portal vein has to form a comfortable C-curve to enter the graft. The slightest stretch resulted in thrombosis in four cases. We also suggest performing the anastomosis at the bifurcation of the splenic and mesenteric vein to allow maximal inflow. In all cases when it occurred, the clot could be extracted from the portal vein, and repositioning of the portal vein provided permanent patency. No graft was lost to portal vein thrombosis, but attention should been given to this surgical detail in further application of LRT.

Hepatic venous outflow obstruction was prevented by appropriate positioning and triangular attachment of the left hepatic vein close to the diaphragm, as we have described.[23] Torsion of the graft occurred in one case, however, and required stabilization of the segment by means of a tissue-expanding prosthesis positioned into the right subdiaphragmatic space. The prosthesis was removed without need for laparotomy after the graft was fixed in the proper position.

The application of reduced-size livers has become an important factor in reducing death while on the waiting list. It has not yet solved the overall organ shortage for liver transplantation. Our results indicate that LRT can provide an excellent graft for the small pediatric recipient at little risk to a relative who wishes to volunteer to be a donor. We speculate, given our current experience, that LRT may have significant advantages over conventional transplantation with cadaver grafts. Certainly there is a strategic advantage. Although performed in elective situations in this protocol, use in children with fulminant hepatic failure could significantly reduce death and morbidity rates. With further use in children with chronic liver disease, LRT could reduce the suffering experienced while waiting for a cadaver donor, which affects patients and families severely, and could eliminate for some the competition for cadaveric organs, which requires children to reach a critical medical status to qualify for a cadaveric organ. There has been a learning curve for both the donor hepatectomy and the recipient operation, so the results of future LRT operations should exceed those realized in this series, which are already very good for this population of small recipients. Furthermore the advantage of LRT with respect to the development of chronic graft rejection and graft longevity have yet to be realized. We believe that our current approach will result in a reduction of complications and improved graft survival.

The success of this program has led to the acceptance of LRT for general clinical application in our institution. If extended to other institutions, our experience should serve to shorten the learning curve and reduce risk to donors and recipients, but teams with little experience in RLT should not seek to undertake LRT, because this procedure is more difficult and places at risk a healthy donor.

References

1. Singer PA, Siegler M, Whitington PF, et al. Ethics of liver transplantation with living donors. N Engl J Med 1989; 321:620–622.
2. Bismuth H, Houssin D. Reduced-size othotopic liver graft in hepatic transplantation in children. Surgery 1984; 95:367–372.
3. Broelsch CE, Neuhaus P, Burdelski M, et al. Orhtotopic liver transplantation of hepatic segments in infants with biliary atresia. Langenbecks Arch Chir 1984; (Suppl):105–109.
4. Broelsch CE, Emond JC, Thistlethwaite JR, et al. Liver transplantation with reduced-sized donor organs. Transplantation 1988; 45:519–524.
5. Singer PA, Lantos JD, Whitington PF, et al. Equipoise and the ethics of segmental liver transplantation. Clin Res 1988; 36:539–545.
6. Otte JB, de Ville de Goyet J, Sokal E, et al. Size reduction of the donor liver is a safe way to alleviate the shortage of size-matched organs in pediatric liver transplantation. Ann Surg 1990; 211: 146–157.
7. Ryckman FC, Flake AW, Fisher RA, et al. Segmental orthotopic hepatic transplantation as a means to improve patient survival and diminish waiting-list mortality. J Pediatr Surg 1991; 26:422–428.
8. Strong R, Ong TH, Pettay P, et al. A new method of segmental orthotopic liver transplantation in children. Surgery 1988; 104: 104–107.
9. Kalayouglu M, D'Alessandro AM, Sollinger HW, et al. Experience with reduced-size liver transplantation. Surg Gynecol Obstet 1990; 171:139–147.
10. Emond JC, Whitington PF, Thistlethwaite JR, et al. Reduced-size orthotopic liver transplantation: use in the management of children with chronic liver disease. Hepatology 1989; 10:867–872.
11. United Network for Organ Sharing: 1990 transplant statistics. News release. April 22, 1991.
12. Emond JC, Whitington PF, Thistlethwaite JR, et al. Transplantation of two patients with one liver: analysis of a preliminary experience with "split liver" grafting. Ann Surg 1990; 212:14–22.
13. Pichlmayr R, Ringe B, Gubernatis G, et al. Transplantation einer Spenderleber auf Zwei Empfanger (Split liver transplantation) Eine neue Methode in derVeiterentwicklung derLebersegment transplantatione. Langenbecks Arch Chir 1989; 373:127–130.
14. Bismuth H, Castaing D, Garden OJ: Segmental surgery of the liver. Surg Annu 1988; 20:291–310.
15. Iwatsuki S, Todo S, Starzl TE. Excisional therapy for benign hepatic lesions. Surgery 1990; 171:240–246.
16. Bismuth H, Houssin D, Michel F. Le risque operatoire des hepatectomies: experience sur 154 hepatectomies: a propos de la communication de M.A. Rohner sur les hepatectomies, seance du 3 Fevrier 1982. Chirurgie 1983; 109:342–348.
17. Whitington PF, Balistreri WF. Pediatric liver transplantation: indications, contraindications, and pre-transplant management. J Pediatr 1991; 118:169–177.
18. Kahn CE Jr, Bae KT, Giger ML, Chen CT. Three-dimensional visualization of the liver from computed-tomography data. In: Proceedings of the first conference on visualization in biomedical computing. Los Alamitos, CA: IEEE Computer Society Press, 1990, pp 447–449.
19. Woodle ES, Thistlethwaite JR, Emond JC, et al. OKT3 therapy for hepatic allograft rejection: comparison of results in adults and children. Transplant Proc 1990; 22:1765–1766.
20. Whitington PF, Emond JC, Whitington SH, et al. Small bowel length and the dose of cyclosporine in children after liver transplantation. N Engl J Med 1990; 322:733–738.
21. Raia S, Nery JR, Mies S. Liver transplantation from live donors. Lancet 1989; ii:497–498.
22. Strong RW, Lynch SV, Ong TH, et al. Successful liver transplantation from a living donor to her son. N Engl J Med 1990; 322:1505–1507.
23. Emond JC, Heffron T, Whitington PF, Broelsch CE. Hepatic vein obstruction: a unique complication of reduced size liver transplantation. Ann Surg (in review).

DISCUSSION

DR. J. VACANTI (Boston, Massachusetts): Dr. Broelsch and his group have again, in an innovative way, attacked one of the major problems in pediatric liver transplantation, that of donor scarcity.

As I read the manuscript last week, I asked myself three questions: (1) Is this procedure medically and surgically successful? (2) Is it medically indicated? and (3) Is it ethically acceptable?

With regard to the first question, I think he has elegantly shown that it is surgically and medically successful. We must remember that this is the first trial, and there is a steep learning curve. Even with that the results in terms of morbidity and mortality rates are as good as any results as it relates to conventional means of pediatric liver transplantation.

Secondly is it medically indicated? This is an area of controversy in the field. Many in the field would say that because there are no children today dying while waiting on the list, that this is excessive therapy, exposing a well donor to harm. I think we must take the broad view, however, and look at the field in general.

In this year there will be approximately 2500 liver transplant operations performed. By contrast, 30,000 people in the United States will die of liver disease. Twenty thousand of those thirty thousand have current indications for transplantation. Therefore as the number of centers offering transplantation increases, the effective donor pool will continue to decrease.

Those of us in the field and those of us who have had an opportunity to visit Chicago know that the pain to the family while waiting for a donor liver is enormous, and there is significant morbidity, because the current allocation system requires that the children be the most ill and have waited the longest. I think we must factor this into the equation.

Finally is it ethically acceptable? I would say that the Chicago group should be commended for the open nature of the continuing debate in this regard.

I would just like to add that many of the points are similar to the debate that occurred with living donor renal transplantation, and this will continue to be discussed.

So in summary I would like to ask Dr. Broelsch what his view is on the medical need, what do you feel the future is of this technique in conjunction with the role of cadaveric whole and reduced size transplantation, as well as liver grafting? And finally do you feel that this will play a role in adult diseases?

I would like to thank the Association for the privilege of discussing this paper.

DR. HENRI BISMUTH (Villejulf, France): I would like to first congratulate Dr. Broelsch for his very interesting paper.

Even if we do not begin with this technique in Europe, I am sure that it will be introduced soon, probably by Dr. Broelsch himself.

Although he has worked with small infants, my questions relate to adult recipients, as the popularity of the reduced size graft in Europe, where an adult liver is used for children, has meant that not only is the adult waiting list longer, but more adults than children are dying while on these lists.

As I see it, there are two main questions about adult living related donations and I would very much like to hear Dr. Broelsch's opinion about these. The first difficulty is the donor himself. If he or she is a child of the recipient, the ethical issues and pressures become extremely difficult. If the donor is a parent of the recipient, however, then age will become a significant problem.

The second difficulty concerns graft volume. A 250- to 300-g graft that is obtained by the safe resection of segments II and III is approximately equivalent to the hepatic remnant after an 80% resection in a normal patient. Although this is of course sufficient for a normal person, the requirements of the graft, principally avoiding postoperative hepatic failure, before the completion of the regeneration process, are likely to require considerable hepatic reserve. The situation may become even more acute if postoperative recovery is complicated by a rejection crisis, which would further reduce the graft capacity and possibly place the patient at significant risk.

So, my question to Christoph Broelsch is, can he suggest any way in which the volume of the graft, and consequent safety margin, might be increased without any additional risk to the donor?

Thank you.

DR. JEREMIAH G. TURCOTTE (Ann Arbor, Michigan): First of all just so you have the general picture from UNOS, there are about 20,000 people waiting for organs in this country on their list. In 1 year, 1800 of the people on the list died. Liver transplant was looked at in detail on sort of a snapshot. Those at risk would be at risk for at least a year, and still not transplanted, and 21% of those patients were at risk for a year and died. Some of them were children.

I know the statement has been made that no children waiting on the list died, but when that is looked at a little more closely it is really not true.

By opening up this potential for additional donor organs, of course, every time an organ is used from a living related volunteer, that means one less organ is needed from the cadaver pool.

The UNOS Ethics Committee has also looked in detail at the ethics of using the living donors as stimulated by the work at Chicago, because this was something new, and they clearly concluded—and this was not surgeons; this is bioethicists and mostly nonphysicians—that this was a perfectly ethical procedure providing the risk is low to the donor and the results are reasonable. But the answers to those questions have awaited Dr. Broelsch's work, and I think he has demonstrated that the risk to the donor is low, and there are several programs in this country who are currently making plans to start a similar program.

Dr. Broelsch had had years of experience in liver surgery, liver transplantation, and especially in reduced grafts, before he started taking this on.

We have recently looked at our own series. When I left Ann Arbor, we had done 299 liver transplants, as of earlier this week, and in approximately a dozen of them we used interposition grafts. And in the children, we have never had one of those thrombose. In fact there is only one in the pool of interpositional graft that have thrombosed. In pediatric liver transplants, arterial thrombosis is a major problem.

So I think what we are hearing from Dr. Broelsch confirms our own bias that perhaps we should be using the aorta as the site of anastomosis for the arterial supply on a more routine basis, especially in children. And we have used both the superiliac as well as the infrarenal position.

I would like to ask him where all of his anastomoses were, and also how he managed the patients' portal vein thromboses.

DR. FRANCIS D. MOORE (Boston, Massachusetts): I would just like to again thank Dr. Broelsch. The slides went by a little fast, and as he closes, I wish he would just give us his bottom line. That is to say, how many of the patients are now doing well with their segmental liver as originally put in?

Secondly it is a tribute to the speed that those people can move that I do not think this subject was mentioned much at all before the Honorary World Symposium on Liver Transplantation in honor of Tom Starzl, which was in Pittsburgh only 6 or 7 years ago.

As to our arterial thrombosis, Alan Burson and I did a small series of liver transplants almost 30 years ago, and we had a lot of trouble with arterial thrombosis. And I wonder if Dr. Broelsch would tell us what anticoagulants he used and how he used them, and whether there is room there to get some nonheparin anticoagulants into these patients?

And finally I would take a gentle disagreement with Dr. Vacanti that this ethical and surgical problem is not in any way analogous to the use of living donors in kidney transplantation. The first successful series of kidney transplantation, which Dr. Murray did, of course, was from living donors, and then for many years our institution and many others used living donors preferentially, and still do.

There are two or three differences therefore. One, everybody—well, almost everybody—has got two kidneys with all their blood supply and ureter and everything intact, and the sacrificial portion on the part of the donor is really very minor. And secondly that really was the way a lot of kidney transplantation began, and before HLA typing was even available, we had a hunch that, of course, the family donors would be the best, and they still are.

So I would hesitate to make an analogy there with a historically very different problem and very different sequence.

DR. CHRISTOPH E. BROELSCH (Closing discussion): Thank you to all the discussants, and to come to the point, Dr. Moore, 15 of all of them are doing fine and well, are at home, and only two are still in the hospital, the recent two retransplants. One of fifteen is at home with a cadaveric retransplant. The two that have been retransplanted are retransplanted from a live donor and are supposedly doing well, because the complications that have occurred from sepsis are manageable at this point.

We have used anticoagulation in the past. I think this is an important point because a coagulopathy in those primary functioning grafts is corrected within hours of the surgery. It is rarely seen in cadaveric transplants in our series, so I think it is a point of treatment where we can improve.

Dr. Turcotte, I appreciate your mentioning the UNOS' status that it might be ethically sound to do this living transplant. I think what we wanted to show in our initial series is that this is technically feasible, and what kind of liver volume we needed to accomplish transplantation, at least in children. And I am glad to hear that for children it is not the major problem we had anticipated.

To finish up with the arterial thrombosis, I think there is room for improvement. From our learning curve, it becomes clear that—and certainly anatomic prerequisite—a donor or donors that we have used should not be used, and that certain grafts, such as inferior mesentery vein graft, should not be used. I think that appropriate choice alone accounts for four of five of all thromboses, and I think in a subsequent series that really can be eliminated.

As far as a portal vein reconstruction is concerned, that can be done with inferior mesentery vein graft, or even with frozen graft. The point is that, with the rotation of the transplant, the portal vein stretches out, and we had to learn that the portal vein really is too long and to swing it around like a C-shape underneath the tissue to fill into the graft. Once we had done that, all the ones we had just obliterated stayed patent without much anticoagulation treatment. So it was a simple technical problem that we encountered.

The potential or the need for the procedure in general, I believe, will remain in pediatric transplantation, but it will expand into adult transplantation, as Dr. Vacanti and Dr. Bismuth have pointed out.

The question we have not addressed yet, because we were scared right from the beginning, is that we had complications with a left hemihepatectomy. We may have been scared too early, and the price we paid is a few arterial thromboses that lead to graft loss. But I think even a left hepatectomy is safe once it has been sort of done routinely, maybe with a Kasai's technique or with other finger pressure techniques. So I believe that the left hemihepatectomy would remove about 40% of the liver, and that would be sufficient for an adult as well, either as a full replacement of the graft, or as a partial replacement of the graft in orthotopic position.

I still think that the future role of segmental transplantation will mainly be in pediatric transplantation and maybe in correction of metabolic diseases. The organ shortage clearly will not be overcome by simply doing living liver transplants in the future, but the organ shortage is being shifted now, and the adults are affected by that, and very few children—although some still die—but very few children die, and that problem has to be addressed.

Unfortunately it cannot be simply addressed by doing split livers, as we presented here in a series of 30. You may have recognized that the results are about 10% to 15% worse than the regular ones, and we actually discontinued that study because we did not want to put our patients into risk for a procedure that technically is not really mature enough.

So the actual solution of doing cadaveric split transplants is not really at hand, and it might have to be done prospectively in elective candidates, as we have done with our present studies.

What to do about the complications and how to finalize it? I think selection of the donors and appropriate surgical reconstruction, prophylaxis, and coagulation is probably the best thing to do to prevent technical failures. I do believe that in the medical future, a certain percentage of pediatric and adult procedures will be done with segmental liver transplant from live donors.

Splanchnic and Cerebral Vasodilatory Effects of Calcitonin Gene-related Peptide I in Humans

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The ability of synthetic human calcitonin gene-related peptide (CGRP I) to act as an arterial vasodilator was tested in healthy men by measuring arterial blood flow parameters in carotid, superior mesenteric, celiac, and femoral vessels. Calculated volume flow was significantly increased (140 ± 21% of basal) in the SMA with a 2-ng/kg/min infusion of CGRP. Carotid artery volume flow increased dose dependently (96 ± 6%, 122 ± 15%, 135 ± 15% of basal, respectively, with 2, 4, or 8 ng/kg/min). With steady-state infusion, carotid and superior mesenteric arterial flow parameters remained significantly elevated for 30 minutes after cessation of peptide administration. Blood pressure was unchanged. Pulse increased dose dependently. Arterial diameters were unchanged, implying activity at the arteriolar level.

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CALCITONIN GENE-RELATED PEPTIDE (CGRP) is a novel peptide resulting from alternative processing of calcitonin gene transcripts.[1] In thyroid C cells, mRNA encoding for calcitonin is produced, whereas mRNA for CGRP predominates in nervous tissues. Calcitonin gene-related peptide is widely distributed in a variety of neuronal tissues in both the central and peripheral nervous systems and is particularly abundant in perivascular nerves.[2] The peptide has been demonstrated immunohistochemically in close association with cerebral and splanchnic vessels in a number of species.[3] The anatomic localization of CGRP within perivascular nerves has suggested that the peptide may have physiologic relevance in the regulation of arterial blood flow.

Initial reports suggest that CGRP may act as a vasodilator in humans. Calcitonin gene-related peptide I, infused intravenously, produces positive inotropic and chronotropic effects, hypotension, and cutaneous vasodilation in conscious subjects.[4] When directly infused into the brachial artery, CGRP has been noted to produce significant and prolonged increases in forearm blood flow.[5] In healthy male subjects, intravenous CGRP I infusion has recently been reported to increase carotid artery pulsatility index, reflecting increased carotid arterial flow rates.[6] Although the *in vivo* actions of CGRP on regional blood flow have not been systematically studied, these reports make CGRP an attractive agent for human investigation.

The aim of the present study was to investigate CGRP vasoactivity in human splanchnic, cerebral, and extremity arterial beds. To this end we used duplex ultrasonography to measure the effects of synthetic human CGRP I *in vivo* on regional arterial flow in conscious subjects. The specific goals were fourfold: (1) to observe changes in regional blood flow in response to varying doses of CGRP, (2) to determine the duration of CGRP effects in differing vascular beds, (3) to compare alterations in splanchnic blood flow caused by CGRP infusion with those produced by a mixed-nutrient meal, and (4) to measure release of circulating somatostatin as a bioassay of CGRP activity.

Materials and Methods

Subjects

Eight healthy men (age range, 22–29 years) served as subjects for the study. All subjects were nonsmoking, nondiabetics without any history of gastrointestinal or vascular disease. No subject was receiving any form of medication. All were of normal weight for height, sex, and age. The study was approved by the Human Subjects Review Committee of the University of Michigan, Ann

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Arbor, Michigan. The investigation was performed in the Clinical Research Center of the University of Michigan.

The studies were performed in three phases. In the first phase, alterations in regional blood flow, blood pressure, pulse, and circulating levels of somatostatin were recorded in response to stepwise increasing doses of CGRP. In the second phase, the duration of CGRP effects on regional blood flow was recorded after steady-state CGRP infusion. In the final study, the splanchnic effects of CGRP were compared with those of a known physiologic stimulant, a mixed-nutrient meal. Subjects underwent each of the studies on separate days. All subjects were studied in the fasted state.

Duplex Ultrasonography

Blood flow measurements were obtained from the celiac, superior mesenteric, common femoral, and common carotid arteries using duplex ultrasonography[7] (Acuson, Mountain View, CA). To image the celiac and superior mesenteric arteries, a 3.5-MHz probe was used; a 7.5-MHz probe was used to image the femoral and carotid arteries. Blood flow velocities were measured by first imaging the artery of interest using the pulsed Doppler component of the scanner. The angle of insonation was maintained below 70 degrees and was kept constant for all measurements within each study. All measurements were recorded on videotape for later review and analysis.

Each subject was positioned supine on an examination table with the head elevated 20 to 30 degrees. Duplex blood flow parameters of peak systolic velocity, mean velocity, end-diastolic velocity, and calculated volume flow were recorded at 10-minute intervals during a 30-minute basal period and then serially at 10-minute intervals during each study. In the second group of studies, performed to determine duration of CGRP effects, measurements were made at more frequent intervals after completion of CGRP infusion. The cross-sectional diameter of each artery also was determined with each measurement. Basal flow parameters exhibited <10% variation with repeated measurements for each individual.

Peak systolic velocity was determined as the maximum flow velocity recorded during each cardiac cycle. End-diastolic velocity was recorded immediately before the next systolic upstroke. Mean velocity was derived by integrating the area under each individual waveform. Volume flow was calculated from the mean velocity and the measured cross-sectional area of the vessel using the formula:

$$\text{Volume flow} = 3.14 \times \tfrac{1}{4}\,(\text{diameter}^2) \times \text{mean velocity}.$$

Left brachial pulse and blood pressure were recorded continuously during each examination. All values were expressed as percentage of corresponding basal measurements.

CGRP Infusion

Synthetic human CGRP I was provided by Celltech Ltd., Berkshire, England. For CGRP dose–response studies, the peptide was reconstituted in a volume of normal saline appropriate for intravenous delivery at 2, 4, or 8 ng/kg/min. After a 30-minute basal period of saline infusion, CGRP was infused at 2 ng/kg/min through a forearm vein for 30 minutes. A second 30-minute infusion of saline then was followed by CGRP infusion at 4 ng/kg/min. After an additional 30-minute control period, the highest dose of CGRP, 8 ng/kg/min, was infused for 30 minutes. Duplex measurements were made throughout the study, and venous blood samples were obtained at 10-minute intervals for assay of plasma somatostatin.

For CGRP steady-state infusion studies, the peptide was infused continuously at 4 ng/kg/min for 60 minutes; the peptide then was stopped. Duplex measurements were obtained at 10-minute intervals during the infusion, and at 1, 2, 3, 5, 10, 15, 20, and 30 minutes after cessation of infusion.

Meal Response

The effects of a mixed-nutrient meal on splanchnic blood flow was investigated using Ensure-Plus (Ross Laboratories, Columbus, OH). The ingested meal contained 240 mL with 13.0 g protein, 12.6 g fat, and 47.3 g carbohydrate. The meal was ingested over 1 to 3 minutes.

Somatostatin Radioimmunoassay

Serum somatostatin levels were measured by radioimmunoassay by a previously described method.[8] Three serum samples were obtained for each CGRP dose interval. In addition all assays were performed in duplicate. The ED_{50} for the radioimmunoassay was 15 fmol/mL.

Statistical Analysis

All data were coded and then recorded in a computer. Data are expressed as group mean ± standard error of the mean (SEM). Changes in blood flow measurements, hemodynamic parameters, and hormone levels were examined using repeated measures analysis of variance. Statistical significance was assumed for p values less than 0.05. Correlations between circulating somatostatin levels and changes in superior mesenteric artery and celiac artery duplex parameters were calculated by linear regression analysis.

Results

CGRP Dose–Response

Incremental increases in CGRP doses were associated with dose-dependent increases in brachial pulse (Fig. 1).

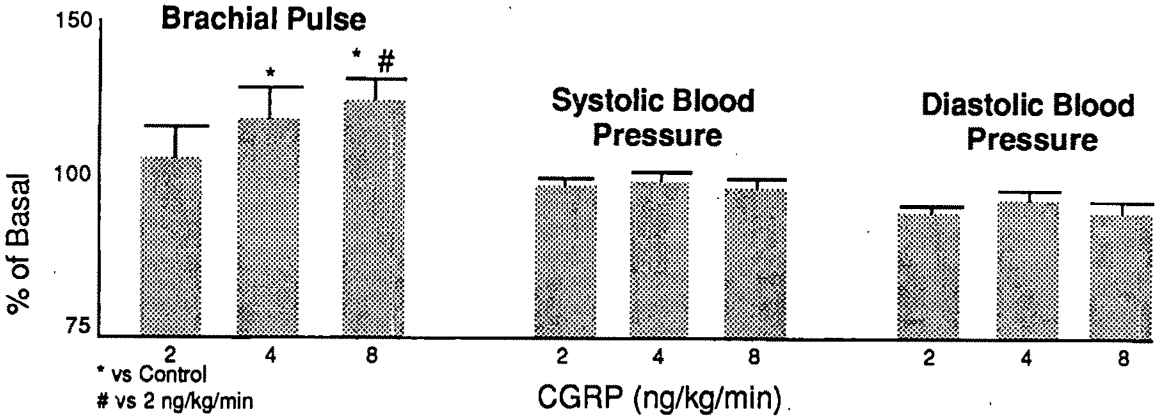

FIG. 1. Hemodynamic parameters in men during dose-response infusions of CGRP.

At 8 ng/kg/min, pulse was increased to 124 ± 5% of basal. No alterations in supine systolic or diastolic blood pressure were recorded. All subjects developed mild facial and truncal flushing with infusion of CGRP at 4 ng/kg/min and moderate to marked flushing at 8 ng/kg/min. Flushing, accompanied by a feeling of suffusion, was considered dose-limiting at this rate of infusion. Facial flushing receded gradually after cessation of CGRP infusion over 20 to 30 minutes. No other systemic or gastrointestinal symptoms were noted.

Superior mesenteric artery (SMA) and celiac artery end-diastolic velocity both were increased during infusion of CGRP at 2 ng/kg/min (141 ± 24% and 143 ± 22% of basal, respectively). For both arteries end-diastolic velocity decreased toward baseline with increasing doses of CGRP (Fig. 2). In contrast carotid artery end-diastolic velocity dose dependently increased with CGRP infusion. At 8 ng/kg/min, carotid artery end diastolic velocity was 156 ± 18% of basal. Femoral artery end-diastolic velocity was increased during CGRP infusion, although to a similar degree for each dose tested (Fig. 2).

Calculated volume flow was significantly increased in the SMA during infusion of CGRP at 2 ng/kg/min. Superior mesenteric artery volume flow decreased toward baseline during the infusion of higher doses of CGRP (Fig. 3). In contrast carotid artery volume flow was unchanged during infusion of CGRP at 2 ng/kg/min, but demonstrated stepwise increases with higher doses of CGRP. Significant increases in all carotid artery duplex parameters were noted during infusion of CGRP at 8 ng/kg/min (Table 1). Volume flow was augmented in celiac and femoral vessels during infusion of CGRP, but the increases did not achieve statistical significance.

Calcitonin gene-related peptide infusion did not result in changes in arterial cross-sectional diameter for superior mesenteric, celiac, carotid, or femoral vessels.

Infusion of synthetic human CGRP I produced modest increments in circulating somatostatin, with significant increases noted at 4 ng/kg/min (121 ± 9% of basal). Linear regression analyses failed to demonstrate significant correlation between circulating levels of somatostatin and alterations in SMA or celiac artery blood flow parameters.

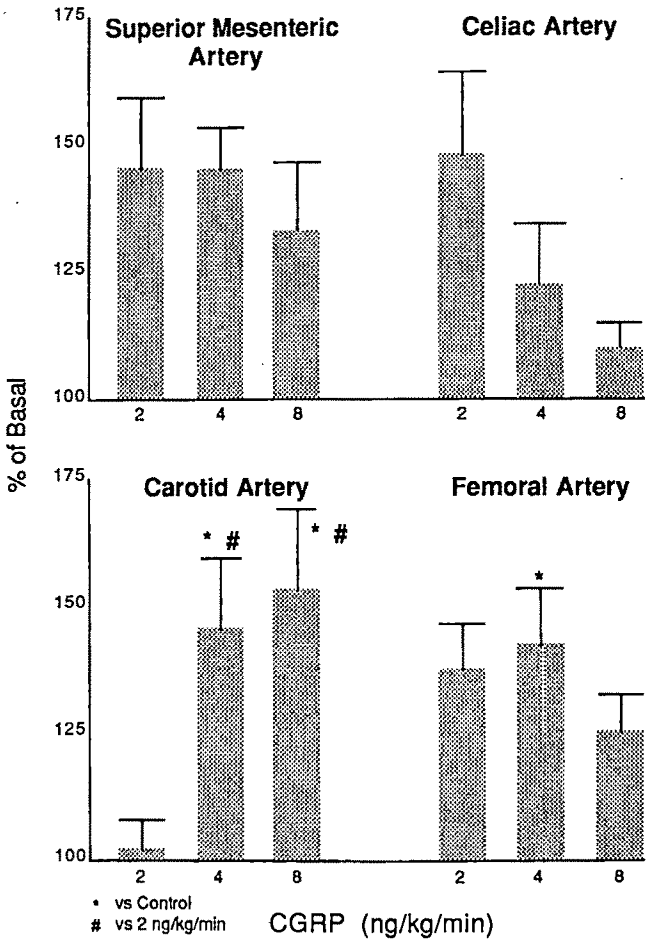

FIG. 2. End diastolic velocity in superior mesenteric, celiac, carotid, and femoral arteries during infusion of synthetic human CGRP I in doses of 2, 4, or 8 ng/kg/min. Data are expressed as percentage of basal measurements.

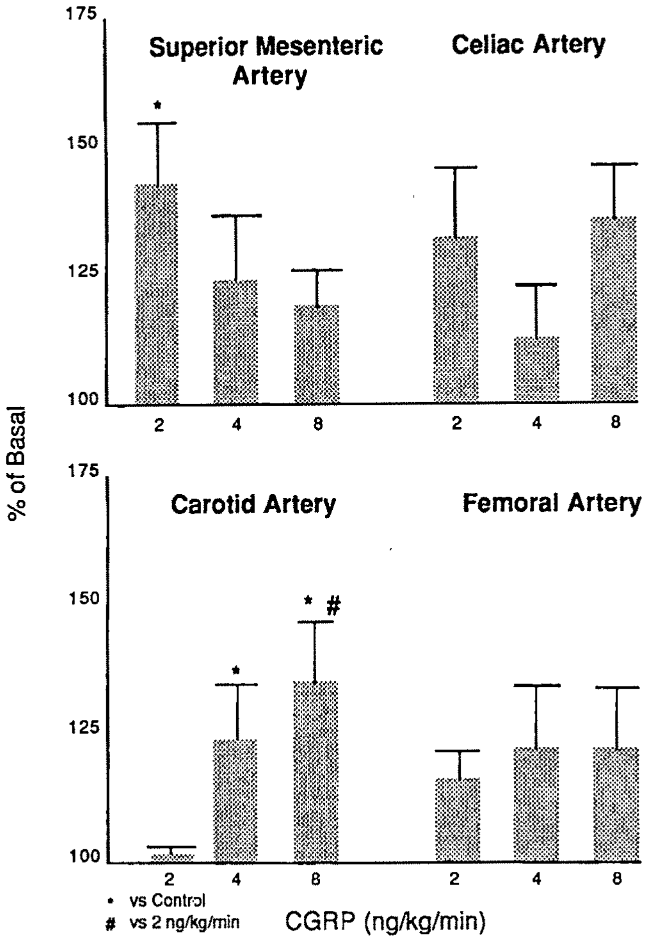

FIG. 3. Calculated volume flow in superior mesenteric, celiac, carotid, and femoral arteries during infusion of synthetic human CGRP I in doses of 2, 4, or 8 ng/kg/min. Data are expressed as percentage of basal measurements.

Steady-state Infusion

The time course of CGRP vasoactivity was examined after continuous infusion of the peptide at 4 ng/kg/min for 60 minutes. Two patterns of reversal of vasodilation were noted (Fig. 4). Calculated volume flow for SMA and carotid artery returned toward baseline slowly, with flow remaining elevated 30 minutes after infusion by 14% and 17%, respectively. Celiac artery and femoral artery volume flow returned to control values rapidly and were at baseline values within 10 minutes of cessation of CGRP infusion.

Meal Ingestion

No alterations in any duplex measurements were recorded for carotid or femoral arteries postprandially. Ingestion of a mixed-nutrient meal, however, produced significant increases in all duplex blood flow parameters for the SMA and celiac artery (Table 2). Superior mesenteric and celiac arteries demonstrated different temporal patterns of response to the ingestion of a meal (Fig. 5). Superior mesenteric artery volume flow was significantly increased relative to baseline within 20 minutes of meal ingestion and remained significantly elevated relative to the fasting state up to 60 minutes postprandially. Celiac artery volume flow was significantly increased by 10 minutes, peaked at 20 minutes, and had returned to basal values by 30 minutes after meal ingestion.

Discussion

The present investigations were performed to test the hypothesis that CGRP is a vasodilatory peptide in humans. To test this hypothesis, we administered synthetic human CGRP I intravenously in healthy male subjects and measured alterations in hemodynamic parameters and in regional blood flow. The results of the investigation suggest that CGRP is a potent vasodilator in humans, but with differing activities in cerebral, splanchnic, and femoral vascular beds. Three observations strongly support the contention that CGRP has vasodilatory actions: (1) Calcitonin gene-related peptide infusion produced dose-dependent increases in carotid artery peak systolic velocity, mean velocity, end diastolic velocity and calculated volume blood flow; (2) SMA volume flow was significantly increased during infusion of CGRP at 2 ng/kg/min, a dose that caused no alteration in peripheral pulse or blood pressure; (3) Carotid artery and SMA volume flow returned to baseline slowly after CGRP infusion and remained above control levels up to 30 minutes after infusion. Additionally in the current study, CGRP infusion was not associated with changes in cross-sectional diameter of the vessels examined. This finding implies that vasodilatory actions are exerted at the arteriolar level within the vascular beds studied.

The response to CGRP infusion varied markedly among the differing arteries studied. Although carotid arterial volume flow increased in a dose-dependent manner, SMA volume flow decreased toward baseline with increasing doses. In addition, unlike carotid and SMA flow, celiac and femoral artery volume flow returned rapidly to basal levels after cessation of CGRP infusion. The mechanisms responsible for these variations were not ex-

TABLE 1. *Percentage Increase over Basal in Carotid Artery Parameters During Infusion of CGRP at 8 ng/kg/min*

Parameter	% of Basal Value
Peak systolic velocity	139 ± 13
End diastolic velocity	156 ± 18
Mean velocity	140 ± 10
Volume flow	135 ± 15

For each parameter, $p < 0.05$ *versus* control and $p < 0.05$ *versus* 2 ng/kg/min.

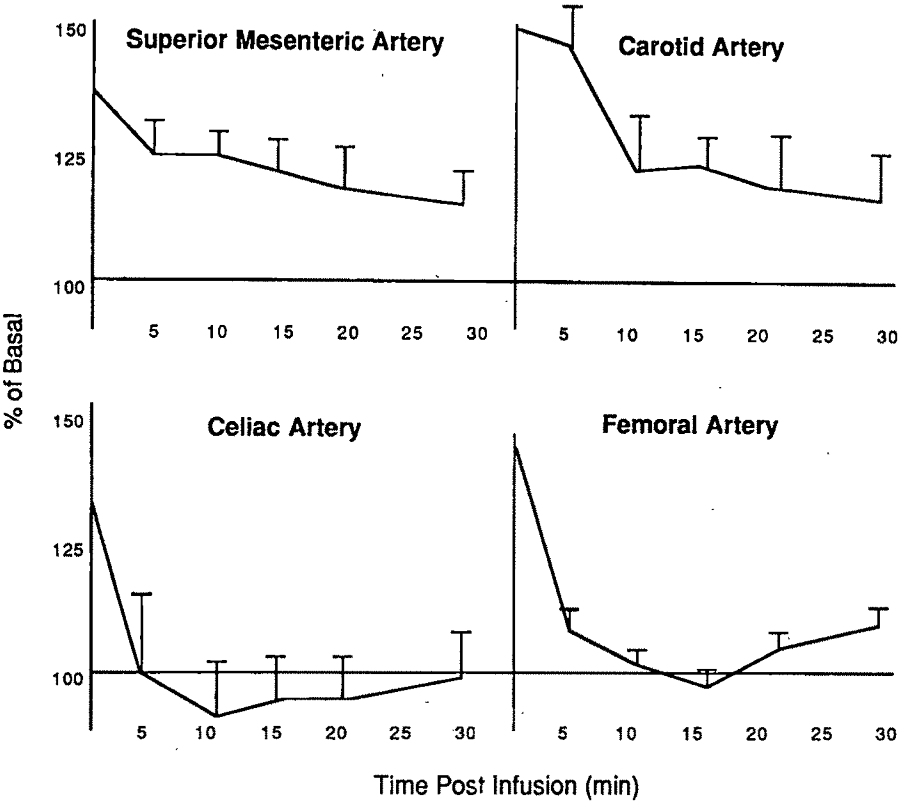

FIG. 4. Calculated volume flow in superior mesenteric, carotid, celiac, and femoral arteries at intervals after cessation of steady-state infusion of 4 ng/kg/min CGRP. Data are expressed as percentage of basal measurements.

amined in the current study, and remain an important area for future investigation.

Although a systematic *in vivo* comparison of CGRP vasoactivity in various vascular beds in humans has not been reported previously, prior observations support the contention that the peptide has vasodilatory properties in man. Intravenous bolus injections of CGRP I have been reported to cause cutaneous vasodilation and to be accompanied by hypotension.[4] Brachial artery injection of CGRP has been reported to cause significant forearm arterial dilation.[5] Intravenous infusion of human CGRP I also has been associated with significant decreases in impedance to carotid artery flow.[6]

In vitro observations of isolated human arteries also indicate that CGRP may possess vasodilatory properties. Calcitonin gene-related peptide caused relaxation of human cerebral arteries precontracted *in vitro* by prostaglandin F_{2alpha}.[9] The peptide has also been reported to dilate human mesenteric arteries *in vitro* that had been pretreated with either prostaglandin or noradrenaline to induce contraction.[10,11] Calcitonin gene-related peptide relaxes, *in vitro,* human skeletal muscle arteries previously exposed to noradrenaline.[12] Caution must be exercised, however, in drawing comparisons between the mechanisms operative *in vitro* and observed *in vivo* vascular responses. The vasoactive properties of exogenously administered CGRP may be modified by secondary release of other vasoactive compounds or by neurally mediated compensatory responses.

TABLE 2. *Maximal Percentage Increase over Fasting Values in Duplex Measurements After Meal Ingestion**

Artery	Peak Systolic Velocity	End Diastolic Velocity	Mean Velocity	Volume Flow
SMA	202 ± 68	389 ± 129	293 ± 76	298 ± 75
Celiac	265 ± 83	228 ± 40	271 ± 82	277 ± 88

* Maximal increases for SMA measurements were noted at 40 min; celiac measurements were maximally increased at 20 min postprandially.
SMA, superior mesenteric artery.

In this regard human CGRP I was demonstrated in the current investigation to stimulate the systemic release of somatostatin, confirming previous observations in other species.[13–15] Levels of circulating somatostatin observed after CGRP infusion did not, however, correlate with alterations in splanchnic vasoreactivity. Exogenously administered somatostatin has been reported to increase mesenteric vascular resistance and to result in decreased mesenteric blood flow.[15,16] Although not assessed directly by the current investigation, it is interesting to speculate that decreased SMA and celiac vasodilation observed during infusion of CGRP at 4 and 8 ng/kg/min might occur secondary to increased local release of somatostatin not reflected in circulating hormone levels.

The most important physiologic stimulant for splanchnic blood flow is, of course, ingestion of a meal. This

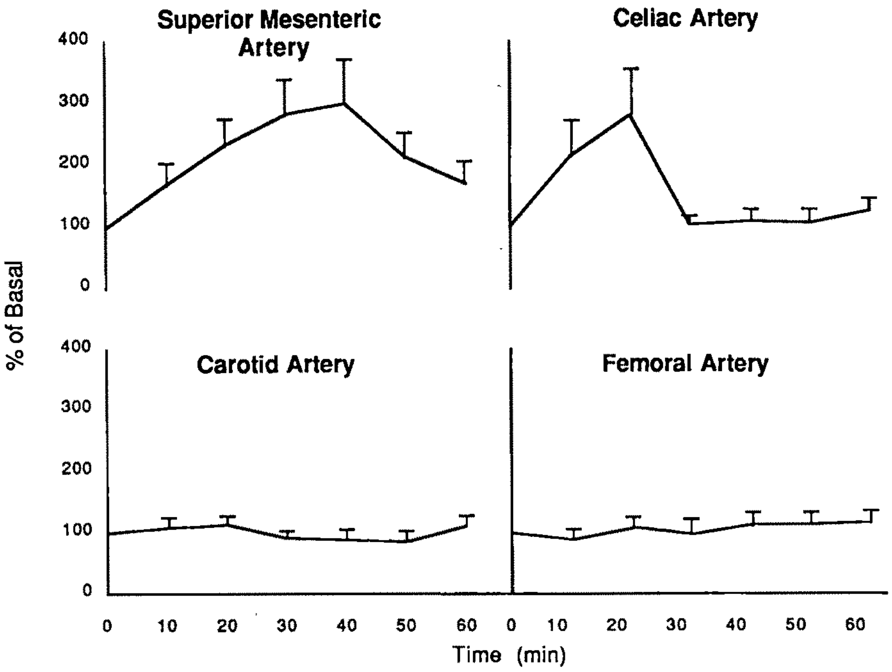

FIG. 5. Calculated volume flow in superior mesenteric, celiac, carotid, and femoral arteries at time intervals after ingestion of mixed-nutrient liquid meal.

investigation confirms previous studies that have used duplex ultrasound to detect postprandial increases in mesenteric blood flow. Celiac artery and SMA duplex parameters increased significantly after ingestion of a mixed-nutrient liquid meal, with maximal effects noted at 20 and 40 minutes, respectively. Increases in splanchnic duplex parameters caused by infusion of exogenous CGRP were lower in magnitude than those produced by a meal. The relevance of this observation to potential physiologic actions of CGRP is limited, however. The local, perivascular levels of CGRP achieved by intravenous infusion can not be determined by currently available techniques. In addition, vasoactive agents, such as somatostatin, released during infusion of exogenous CGRP, may alter vascular responsiveness but may not be relevant to the *in situ* actions of CGRP. The physiologic role of CGRP in postprandial modulation of mesenteric blood flow awaits the development of methods to measure peptide concentrations in perivascular tissues.

Finally the potent cerebral vasodilatory actions of CGRP make it a potential therapeutic agent for disorders characterized by cerebral vasoconstriction.

References

1. Amara SG, Jonas V, Rosenfeld MG, et al. Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. Nature 1982; 298:240–244.
2. Sasaki Y, Hayashi N, Kasahara A, et al. Calcitonin gene-related peptide in the hepatic and splanchnic vascular systems of the rat. Hepatology 1986; 6:676–681.
3. Feher E, Burnstock G, Varndell IM, Polak JM. Calcitonin gene-related peptide-immunoreactive nerve fibers in the small intestine of the guinea-pig: electron-microscopic immunocytochemistry. Cell Tissue Res 1986; 245:353–358.
4. Franco-Cereceda A, Gennari C, Nami R, et al. Cardiovascular effects of calcitonin gene-related peptides I and II in man. Circ Res 1987; 60:393–397.
5. McEwan JR, Benjamin N, Larkin S, et al. Vasodilatation by calcitonin gene-related peptide and by substance P: a comparison of their effects on resistance and capacitance vessels of human forearms. Circulation 1988; 77:1072–1080.
6. MacDonald NJ, Butters L, O'Shaughnessy DJ, et al. A comparison of the effects of human alpha calcitonin gene-related peptide and glyceryl trinitrate on regional blood velocity in man. Br J Pharmacol 1989; 28:257–261.
7. Moneta G, Taylor DC, Helton WS, et al. Duplex ultrasound measurement of postprandial intestinal blood flow: effect of meal composition. Gastroenterology 1988; 95:1294–1301.
8. Seal A, Yamada T, Debas H, et al. Somatostatin-14 and somatostatin-28: clearance and potency on gastric function in dogs. Am J Physiol 1982; 243:97–102.
9. Mejia JA, Pernow J, von Holst H, et al. Effects of neuropeptide Y, calcitonin gene-e/related peptide, substance P, and capsaicin on cerebral arteries in man and animals. J Neurosurg 1988; 69:913–918.
10. Tornebrandt K, Nobin A, Owman C. Contractile and dilatory action of neuropeptides on isolated human mesenteric blood vessels. Peptides 1987; 8:251–256.
11. Marshall I, Al-Kazwini SJ, Holman JJ, Craig RK. Human alpha-calcitonin gene-related peptide (CGRP) is a potent vasodilator in human mesenteric vasculature. Br J Clin Pharmacol 1988; 26: 691–695.
12. Pernow J. Actions of constrictor (NPY and endothelin) and dilator (substance P, CGRP, and VIP) peptides on pig splenic and human skeletal muscle arteries: involvement of the endothelium. Br J Pharmacol 1989; 97:983–989.
13. Dunning BE, Taborsky GJ. Calcitonin gene-related peptide: a potent and selective stimulator of gastrointestinal somatostatin secretion. Endocrinology 1987; 120:1774–1781.
14. Helton WS, Mulholland MW, Bunnett NW, Debas HT. Inhibition of gastric and pancreatic secretion in dogs by CGRP: role of somatostatin. Am J Physiol 1989; 256:G715–G720.
15. Mulholland MW, Garcia R, Garcia I, et al. Inhibition of pancreatic exocrine secretion in the rat by calcitonin gene-related peptide: involvement of circulating somatostatin. Endocrinology 1989; 124:1849–1856.

DISCUSSION

DR. HAILE T. DEBAS (San Francisco, California): After almost two decades of physiologic and biochemical studies of gastrointestinal hormones, investigators are now asking whether the action of these peptides can be exploited for clinical advantage, both in the diagnosis and therapy of human disease. The findings of the University of Michigan group suggests that CGRP promises to have important clinical application in modifying cerebral vascular circulation.

Calcitonin gene-related peptide, the peptide they have studied, is one of over 30 neuropeptides found in the gastrointestinal tract. Calcitonin gene-related peptide neurons, as mentioned by Dr. Mulholland, form a rich network of nerves around small vessels, not only in the gut, but also in the cerebral vascular bed, and in the myocardium. Calcitonin gene-related peptide is present also in 80% of the sensory neurons of the stomach and duodenum, and with substance P, accounts for the major sensory innervation of the gastrointestinal tract.

The application of mild irritants to the gastric mucosa is known to result in protection of the stomach from the effects of subsequent application of stronger irritants, a phenomenon that some have referred to as adaptive cytoprotection. We now know that irritants are also sensed by CGRP neurons in the gastric mucosa, that CGRP is released from these nerves and also from perivascular nerves, and that CGRP, perhaps mainly through its potent vasodilatory effect, mediates mucosal protection.

The hemodynamic effects of CGRP shown by Dr. Mulholland also appear to be due to the effect of the peptide on small vessels. The powerful vasodilatory effect of CGRP on mesenteric and cerebral circulation suggests a potential future application of CGRP in mesenteric and cerebrovascular ischemia.

I would suggest also that CGRP may be important in regulation of blood flow in the myocardium, and studies are needed to evaluate this hypothesis and to see if CGRP infusion could protect against ischemic injury of the myocardium.

With respect to gastrointestinal application of CGRP, I would recommend to Dr. Mulholland a study of gastric mucosal protection.

I have three questions for Dr. Mulholland. First you observed maximal changes in superior mesenteric artery end celiac artery end-diastolic velocity and volume flow with the lowest dose of CGRP employed. This suggests that all the doses you have studied may have been close to the supramaximal dose. This is particularly important with CGRP, because with the high doses it releases somatostatin, which may inhibit its action.

Second the dose-response studies were performed in single experiments where ascending doses of CGRP were given, separated by 30-minute basal periods. Do you know whether the vascular effects of CGRP are susceptible to tachyphylaxis? I ask this question because in a study we performed on the effect of substance P on pepsinogen release, two identical doses were given separated by 60 minutes, different results were obtained, the second dose always giving a lower response. We do not know whether this phenomenon is due to downregulation of the substance P receptor or to some other mechanism.

And finally do you know if CGRP crosses the blood-brain barrier? If not how does it affect cerebral circulation?

Again I wish to congratulate Dr. Mulholland and his co-workers for an excellent study with important potential therapeutic application.

DR. ROBERT W. BARNES (Little Rock, Arkansas): Dr. Mulholland and his colleagues have added meaningful data to an enlarging body of knowledge about the physiologic effects of calcitonin gene-related peptide, or CGRP, a potent vasodilatory peptide localized to perivascular nerves. I believe that these *in vivo* studies in normal volunteers were rigorously performed, and I can find little to fault regarding their methodology.

It would have been helpful if they had included an evaluation of the peripheral hemodynamics in the hands or feet in addition to the gross assessment of femoral artery blood flow, particularly because CGRP release has been noted to be deficient in the hands of patients with Raynaud's syndrome, as reported by Shawkets et al. in Lancet 1989; ii:1354–1357 and Dowd PM et al. in Lancet 1990; ii:1014.

Presumably the absence of a normal physiologic vasodilatory neuropeptide in such patients may lead to the unopposed vasoconstrictive effect of endothelin-1, which has been found by immunofluorescence in dermal capillary loops of normal human digital skin, which may explain the "local fault" hypothesis of Raynaud's syndrome described by Lewis and Pickering over 50 years ago.

I would be interested in knowing if the authors have had the opportunity to study the therapeutic effect of CGRP on any patients with cold sensitivity states?

Furthermore could this neuropeptide play any role in the cause of vasodilatory states, including migraine, the cerebral hyperperfusion syndrome that occasionally follows carotid endarterectomy, or the rather rare foot hyperemic condition of erythromelalgia?

Finally do the authors believe that any analogs of CGRP might be developed that would permit oral therapy for conditions that may be caused by a deficiency of this interesting neuropeptide?

DR. RICHARD A. PRINZ (Maywood, Illinois): Dr. Christopher Joyce in our laboratory has been very interested in this potent vasodilator and has evaluated the effects of CGRP in dogs on coronary artery blood flow. He has shown a significant increase in coronary flow and a decrease in coronary resistance in a dose-response fashion with injection of CGRP (*J. Surg Res* 1990; 49:435–440). He has taken these findings to our intensive care unit and looked at patients who have hyperdynamic sepsis. In patients who have a septic cardiovascular picture with decreased systemic vascular resistance, decreased mean arterial pressure, elevated heart rate, and cardiac output, CGRP levels are 7.5 times higher than in control subjects (*Surgery* 1990; 108:1097–1101). I would like Dr. Mulholland to speculate on the potential pathophysiologic effects of CGRP in man. I also noticed that his flow data was in percent change. I wonder if he could give us some idea of the absolute flow in these vessels and the absolute changes that were seen.

DR. EDWIN L. KAPLAN (Chicago, Illinois): I want to ask whether any other neuropeptides, such as parathyroid hormone, have been tried. About 20 years ago, Charbon found that the infusion of parathyroid hormone greatly increased hepatic artery blood. Dr. Peskin and I repeated these experiments and found similar results. This may be a fruitful area of study.

DR. JAMES THOMPSON (Galveston, Texas): Parathyroid hormone is a potent vasidilator, and PTHRP, the related peptide, is even stronger. These studies have been done by Carrie Cooper in our group.

I would like to ask Dr. Mulholland if he has done any of these studies in which they have measured the EEG response to stimuli, say a flickering light, with and without application of CGRP to increase the cerebral flow, because if it works, I think I might start taking some of that stuff.

DR. MICHAEL W. MULHOLLAND (Closing discussion): Well I would like to thank all the people for their thoughtful comments.

Dr. Debas asked several questions about how dosing in the study was performed, and our observation of decreasing responsiveness in splanchnic vessels with increases in dose. I think it is likely to be one of two things. First, in our study design, we gave doses in incremental, increasing order on the same day. We gave the lowest dose first because of concerns about safety. We were concerned that by giving the highest dose or in a random order, we might have hypotension develop. That was not observed. Because of this dose order, our results could be influenced by tachyphylaxis or receptor downregulation.

A second equally plausible explanation is that the peptide stimulates some secondary action: for example release of local somatostatin, which then acts as a vasoconstrictor. Calcitonin gene-related peptide is the most potent releasor of systemic somatostatin known. That is a possibility that we investigated in our study. Systemic somatostatin increased only about 20% in humans with CGRP-1, and correlation of increments in somatostatin with decrements in blood flow showed no relationship one to the other. But I am sure CGRP activates other secondary phenomena as well.

Dr. Barnes asked—and several of the other discussants asked—about its relationship to pathophysiologic states or even normal physiology. There is not much known about this issue, although what is known hints that CGRP could be important. For example CGRP in the peripheral blood is increased in the third trimester of pregnancy. Calcitonin gene-related peptide is increased in septic shock. The peptide is decreased by immunocytochemistry in vasospastic phenomena; for example, Raynaud's disease.

The relationship of circulatory CGRP levels, though, to physiology, I think, is very tenuous. Calcitonin gene-related peptide is a neuropeptide. I think its actions are entirely local. What gets into the serum, I think, is probably an overflow phenomenon, and I would doubt that it has a direct relationship to what happens at the local level.

Several of the discussants asked about therapeutic uses for CGRP. I think it is very likely to be useful in the next few years. Surgeons that are most likely to use the peptide are going to be neurosurgeons, because it is a very potent vasodilator for intracerebral vessels, especially in states where they are precontracted or vasospastic. I think cardiac surgeons or cardiologists are also likely to be using it.

A couple of the discussants asked about absolute levels of volume-flow measurements. I am a little bit reluctant to discuss those, because there are a lot of assumptions in making absolute volume-flow measurements that make me a little bit uncomfortable.

Expression of Neurotensin Messenger RNA in a Human Carcinoid Tumor

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Neurotensin (NT), a distal gut peptide, has important regulatory and trophic effects throughout the gut; however the intracellular mechanisms that regulate the gene expression and release of human NT are not known. The purpose of this endeavor was to study a functioning human pancreatic carcinoid cell line (called BON) *in vitro* that expresses the NT gene, and to study the effect of the cyclic adenosine monophosphate (cAMP) signal-transduction pathway on the expression and release of human NT. RNA was prepared from BON cell line (which has been established in this laboratory); the RNA was analyzed for NT mRNA expression by Northern hybridization with a complementary DNA probe. RNA blot analysis demonstrated that the NT gene is expressed in BON and is transcribed to two mRNAs of 1.0- and 1.5-kb sizes. In the second part of this study, BON cells were treated with either forskolin (FSK), which increases intracellular levels of cAMP, or with serotonin (5-HT), which reduces cAMP in BON cells. Forskolin produced a dose-dependent increase in NT peptide release and, furthermore, FSK (10^{-6} mol/L) rapidly increased NT mRNA abundance 1 hour after addition; conversely, 5-HT (10^{-5} mol/L) decreased NT mRNA at 1 hour. Neurotensin mRNA levels returned to control values by 3 hours after either FSK or 5-HT, which suggests that the transcript half-life for NT is relatively short. These findings show that the expression and peptide release of human NT is mediated, in part, by the cAMP signal-transduction pathway. Our human carcinoid cell line will provide a useful model to study the *in vitro* regulation of NT gene expression and peptide release.

NEUROTENSIN (NT) IS A tridecapeptide with greatest abundance in the central nervous system and gut (particularly distal small intestine).[1-4] Physiologic actions of NT in the gut include stimulation of pancreatic secretion and colonic motility[5,6] and inhibition of gastric acid secretion and small bowel motility.[7,8] Recently studies have shown that NT stimulates the growth of the normal small bowel,[9] gastric antrum,[10] and pancreas.[10,11] We have found that NT can prevent the small bowel mucosal atrophy associated with feeding rats an elemental diet[12] and that this effect appears to be mediated in part by a direct systemic effect.[13] In addition to its trophic effects on normal gut tissues, NT has been identified in human small cell lung cancers[14] and stimulates the growth and intracellular calcium mobilization in a number of these cancer lines *in vitro*.[15,16] Collectively these studies suggest a possible role for NT in the autocrine or paracrine growth of both normal and malignant tissues. Studies that examine the molecular mechanisms of NT gene expression and release will facilitate better understanding of the intracellular mechanisms responsible for these trophic effects; efforts to date have been hampered by the lack of an established human cell line that expresses the NT gene.

We have established and characterized a human foregut carcinoid tumor line (BON) in athymic nude mice[17] and in tissue culture. The BON line possesses receptors for gastrin and somatostatin, and produces and secretes serotonin (5-hydroxytryptamine [5-HT]),[18] pancreastatin,[19] and chromogranin A.[20] To our knowledge no other long-term functioning cell line from human carcinoid tumors has been described. BON has proven to be a useful model to study carcinoid tumor biology[18,21] and to examine the effects of various chemotherapeutic agents on the inhibition of carcinoid tumor growth.[22,23] BON produces a variety of hormones and amines; however the presence of the NT gene in BON has not been determined previously.

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The purpose of this study was to determine whether BON cells express the gene for NT and, if so, to begin to define the molecular mechanisms responsible for the gene regulation and release of human NT.

Materials and Methods

Maintenance of BON in Tissue Culture

BON cells are maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY) and F12K (Gibco) in a 1:1 ratio supplemented with 5% (vol/vol) fetal calf serum (FCS) (Hyclone Laboratories, Logan, UT) in a humidified atmosphere of 95% air and 5% CO_2 at 37 C. Stock cultures are passed at a 1:2 ratio when cells reach 80% confluence. Cell cultures are routinely monitored for mycoplasma contamination, and no mycoplasma growth has been detected.

In the present study, we have used BON cells in passages 9 through 12.

Phase Contrast and Electron Microscopy

For phase contrast microscopy, BON cells were grown for 4 days in DMEM/F12K + 5% FCS and photographed by means of a Nikon (Diaphot-TMD) inverted microscope.

For electron microscopy BON cells (1×10^5) were grown on Millicell-CM (Millipore) filters in DMEM/F12K + 5% FCS for 4 to 7 days. Cells (grown as monolayers) on the filters then were fixed in half-strength Karnovsky's fixative for 4 to 6 hours; they were processed routinely and embedded in epoxy resin (Epon). Sections (70 nm) were stained with uranyl acetate and lead citrate and examined with a Phillips 410 electron microscope (Philips Medical Systems Inc., Shelton, CT).

Neurotensin Radioimmunoassay

Measurement of NT levels in incubation media and sonicated cells was carried out using a specific radioimmunoassay (RIA) established in our laboratory and previously described in detail.[3]

RNA Preparation, Northern Blot Analysis, and Labeling

Total RNA was prepared using the acid guanidinium isothiocyanate-phenol-chloroform procedure.[24] Polyadenylated (Poly $[A]^+$) RNA was selected by oligo(dT) cellulose (Collaborative Research Inc., Bedford, MA) column chromatography. The final RNA concentration was quantified by measuring its absorbance at 260 nm.

For Northern blot analyses, either 20 μg total RNA or 5 μg poly $(A)^+$ RNA was separated on a 1.2% agarose-formaldehyde gel. Size-fractionated RNA then was transferred to nitrocellulose filters by capillary action. After transfer filters were baked for 2 hours in a vacuum oven at 80 C.

^{32}P-CTP (New England Nuclear Research Products, Boston, MA)–labeled antisense (cRNA) probes were synthesized.[4] The canine NT/neuromedin N cRNA probe was synthesized from a 729-bp cDNA insert (NT-8) subcloned into the *Eco*RI site of pGEM 4 vector.[25] Complementary RNA probes corresponding to the cyclophilin gene (1B15) were synthesized from a 680-bp cDNA insert in SP65-1B15.[26] This gene is constitutively expressed in BON and was used to control for RNA loading.

Hybridization and posthybridization washes were performed as described previously,[4] and after washes were completed, filters were blotted dry and exposed to XAR-5 x-ray film (Eastman Kodak, Rochester, NY) in the presence of intensifying screens at −70 C.

The hybridization signals on the blots were analyzed quantitatively using a Bio Image Visage 60 densitometer (Bio Image, Ann Arbor, MI).

NT Release Experiments

BON cells (5×10^5) were harvested by trypsin (1:250, Gibco) and plated into 35-mm tissue culture dishes in DMEM/F12K + 5% FCS. After a 2-day incubation, cells were washed twice with 2 mL oxygenated Krebs-Ringer bicarbonate buffer (KRBB, pH 7.4) containing HEPES (10 mmol/L), bovine serum albumin (BSA) (0.1%), glucose (2.5 mmol/L), and incubated for a further 30 minutes in fresh KRBB containing forskolin (FSK, 10^{-8} to 10^{-6} mol/L) (Sigma Chemical Co., St. Louis, MO), prepared in 100% ethanol. Control cells received the same amount of ethanol, and the final concentration of ethanol in the medium did not exceed 0.1%. After this incubation period, media were saved for measurement of NT levels by RIA. Cells were scraped and sonicated for 30 seconds using a sonic dismembranator (Model 300, Fisher Sonicator) for measurement of intracellular NT levels. All experiments were performed three times using six dishes/treatment; representative data are shown.

Results are calculated as percent fractional release (amount of NT secreted into the medium × [amount of NT secreted into the medium + amount of NT present in the cell extract]$^{-1}$ × 100) and are expressed as percent of control.

Neurotensin mRNA Induction Experiments

BON cells (5×10^6) were grown in monolayer culture in DMEM/F12K medium that was supplemented with 5% FCS in T150 culture flasks for 48 hours. After this incubation period, cells were approximately 70% confluent; the medium was changed to DMEM/F12K with 0.5% FCS for 24 hours, at which time FSK (10^{-6} mol/L) or 5-HT (10^{-5} mol/L) was added to the culture medium.

At the indicated times after addition, the cells were washed with phosphate-buffered saline (PBS) and lysed with 4 mol/L guanidinium isothiocyanate. The cell lysate was frozen at −70 C until RNA extraction.

Statistical Analysis

Results are expressed as the mean ± standard error of the mean (SEM) and analyzed using Student's unpaired t test at the 0.05 level of significance.

Results

Microscopic Studies

Phase-contrast microscopy. BON cells in culture are composed of polygonal cells growing in a cobblestone fashion. The cells are found to extend long cytoplasmic processes resembling dendrites, similar to other cells of neuroendocrine differentiation (Fig. 1).

Electron microscopy. BON cells grow in monolayers and bilayers. They exhibit abundant cytoplasm with a variety of organelles such as mitochondria, profiles of endoplasmic reticulum, pinocytotic vesicles, and numerous membrane-bound secretory granules. The granules are of varying sizes and concentrated at the poles of cytoplasmic processes. The nuclei exhibit irregular contours with one or two inconspicuous nucleoli. The cell-attachment junctions are poorly developed, and the apical surfaces of the cells exhibit numerous microvilli. Production of basement membrane is not apparent (Fig. 2).

Northern Blot Analysis of NT mRNA

Figure 3 shows the Northern blot analysis of NT mRNA detected in BON cells and normal ileal mucosa obtained from a patient at operation. Neurotensin mRNA transcripts of approximately 1.0- and 1.5-kb sizes were demonstrated in both samples after high-stringency washes.

Neurotensin Peptide Release After Addition of Forskolin

To determine whether cyclic adenosine monophosphate (cAMP) affects the release of NT from BON, FSK, which increases intracellular cAMP, was added to the incubation medium. Forskolin stimulated NT release from BON cells in a dose-dependent fashion (Fig. 4). Forskolin (10^{-6} mol/L) produced a maximal 51% increase in NT fractional release compared with control values.

Northern Blot Analysis of NT mRNA After Addition of FSK or 5-HT

We next investigated changes of NT mRNA levels in response to FSK or 5-HT in BON cells. Forskolin (10^{-6}

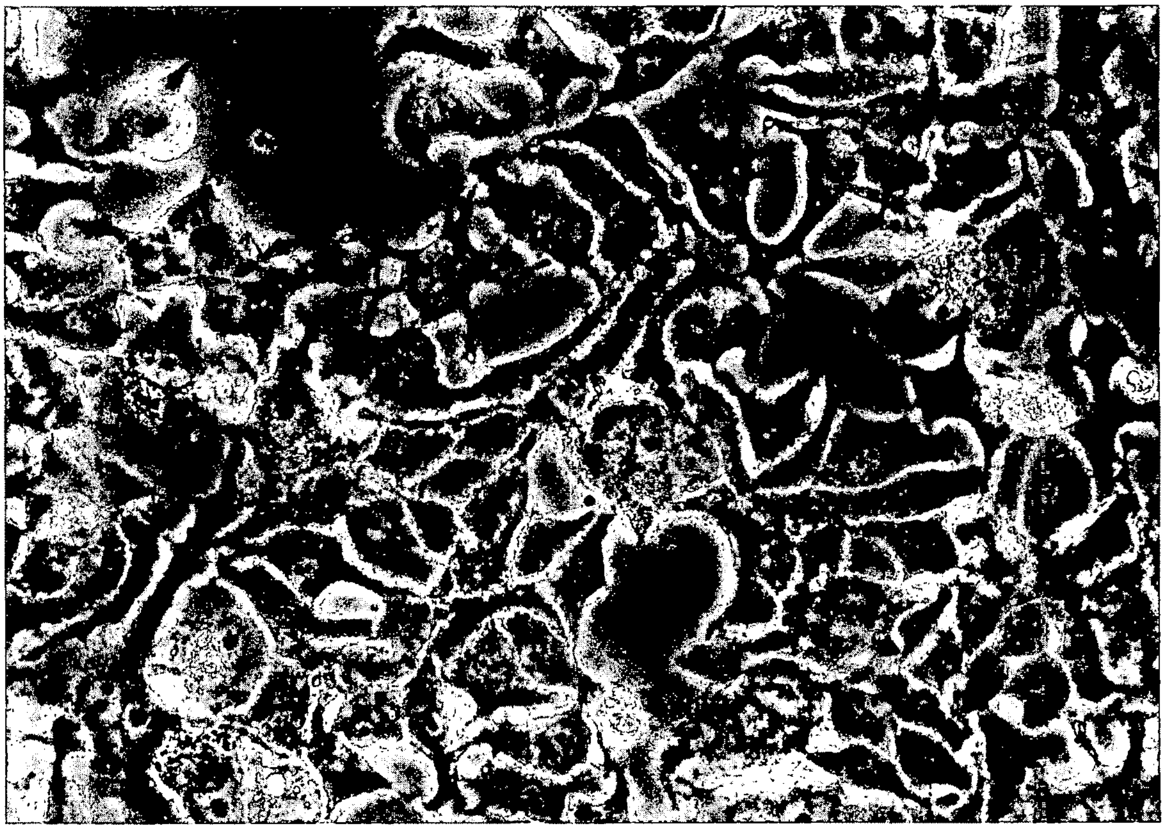

FIG. 1. Phase contrast photomicrograph of BON cells showing long cytoplasmic processes resembling dendrites (original magnification, ×200).

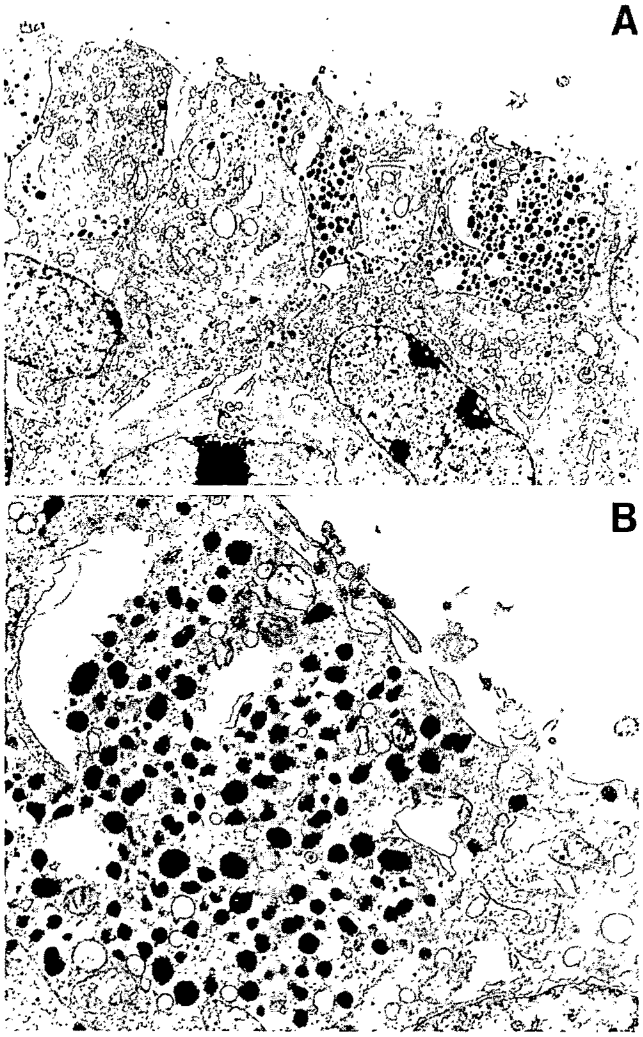

FIG. 2. Electron micrograph of BON cells showing abundant dense secretory granules. (A, original magnification, ×7000) (B, original magnification, ×25,000).

mol/L) was added to the medium, and after 1 and 3 hours, total RNA was extracted and subjected to Northern analysis. Figure 5 shows the basal and cAMP-stimulated NT mRNA levels in BON cells. One hour after addition, there was a 2.4-fold increase in NT mRNA abundance; however at 3 hours, NT mRNA levels had returned to control values. Conversely 5-HT (10^{-5} mol/L), which decreases intracellular cAMP in BON, decreased NT mRNA at 1 hour (Fig. 6). Values rapidly returned to control values by 3 hours (data not shown).

Discussion

In this study we report, for the first time, the long-term establishment of a human carcinoid cell line (BON) *in vitro* that expresses the gene for neurotensin at a relatively high level. Increasing cAMP by adding FSK increases NT gene expression and fractional release. Conversely inhibiting cAMP in BON cells by adding 5-HT decreases NT mRNA expression.

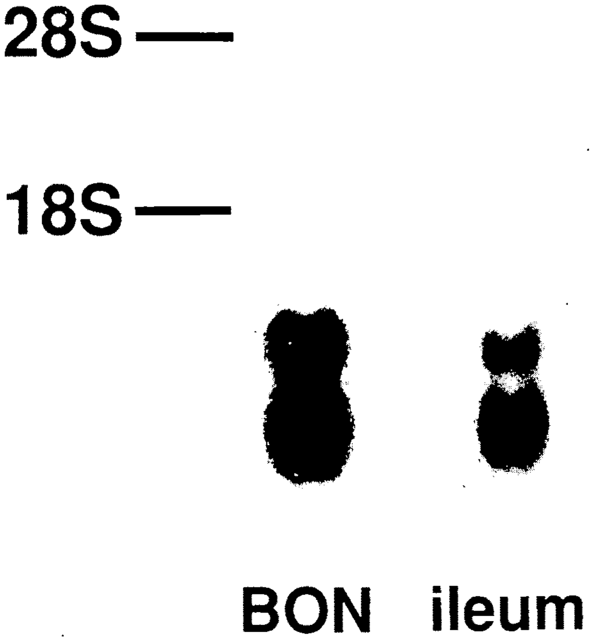

FIG. 3. Expression of NT mRNA in BON and human ileum. Five micrograms of poly (A) + RNA from BON cells and ileal mucosa was analyzed by the Northern blot method. The blots were probed with a canine NT cRNA and were washed at high stringency.

The BON tumor line was established 4 years ago in our laboratory from a peripancreatic lymph node metastasis of a pancreatic carcinoid tumor. BON has remained histologically identical to the original patient tumor and has also retained membrane receptors for both gastrin and somatostatin.[17] Characteristic of the foregut carcinoid, BON cells are multihormonal; previously we have described the production and release of chromogranin A

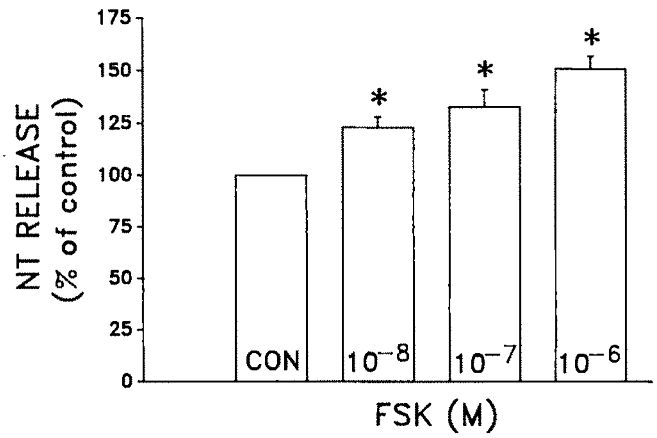

FIG. 4. Effect of FSK (10^{-8} to 10^{-6} M) on NT release from BON cells. Error bars indicate SEM of six plates, and data represent results from three separate experiments. NT fractional release was determined (as stated in Materials and Methods) and was expressed as percent of control. *$p < 0.05$ *versus* control.

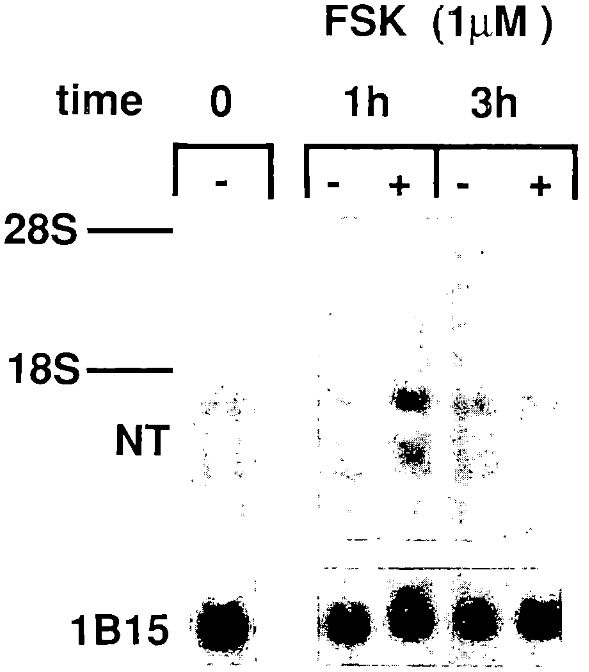

FIG. 5. Expression of NT mRNA in BON cells after addition of FSK (1 μM). BON cells were harvested at the indicated times, and total RNA (20 μg/lane) was extracted. Blots were probed with the canine NT cRNA and then stripped and reprobed with 1B15 to control for loading.

and pancreastatin from BON.[19,20] In addition BON produces and secretes 5-HT both basally[18,21] and in response to gastrin[18]; furthermore we have found that 5-HT is an autocrine growth stimulator for BON cells in culture.[21] We have also used this tumor line for *in vivo* studies. BON cells, when injected into athymic nude mice, will produce discrete tumors[17,22,23]; these tumors have been a useful model to study the effects of various novel chemotherapeutic agents.[22,23]

The gene for NT/neuromedin N is highly conserved in mammalian species and contains four exons separated by three introns.[25,27] The coding region for NT and neuromedin N is located in tandem position on exon 4.[27] Using high-stringency conditions, we have used a canine cDNA probe, cloned by Dobner and colleagues,[25] to probe RNA samples from BON cells and human ileum, which has the highest abundance of gut NT.[3,4] We found that BON expresses the gene for NT and, similar to the findings that we[4] and others[25,27,28] have reported, NT transcribes to two mRNA species of approximately 1.0- and 1.5-kb sizes with the 1.0-kb the predominant transcript. Similar transcript sizes were noted in the ileum.

By means of a variety of techniques, including Northern hybridization, RIA, and high-pressure liquid chromatography, NT has been found in operative specimens of endocrine tumors of the gastrointestinal tract[28,29] and lung.[14] In addition other cell lines, derived from rat endocrine tumors, have been established. These cell lines express the NT gene and secrete NT peptide and include a rat pheochromocytoma (PC12)[30–32] and a rat C-cell–derived tumor line (44-2C).[33,34] Our present study is, to our knowledge, the first to report of the establishment of a human NT-producing carcinoid cell line. Using this unique tumor line as an *in vitro* model, we hope to expand on our previous *in vivo* studies and begin to define the intracellular mechanisms responsible for the regulation of NT expression and peptide secretion.

The transcription of eukaryotic genes is mediated through the activation of intracellular second-messenger pathways that in turn activate specific proteins that bind to promoter elements located in the 5′-flanking region of the gene.[35–38] In our present study, we have examined the effect of the cAMP signal transduction pathway on the expression of human NT mRNA and NT peptide release. Cyclic AMP mediates stimulation of a variety of eukaryotic genes through a conserved cAMP response element (CRE),[36,39] and, recently, Kislauskis and colleagues[27] have found that the rat NT-gene promoter contains a DNA

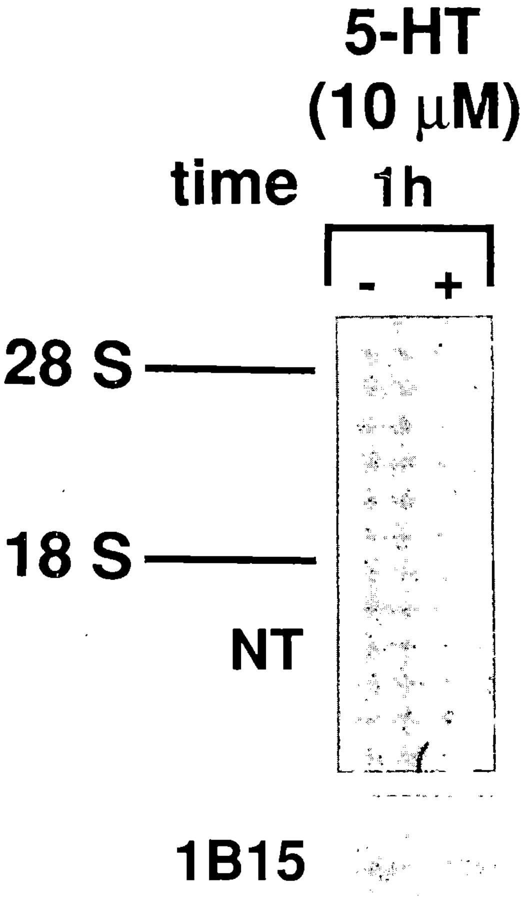

FIG. 6. Expression of NT mRNA in BON cells after addition of 5-HT (10 μM). BON cells were harvested at 1 hour, and total RNA (20 μg/lane) was extracted. Blots were probed with the canine NT cRNA and then stripped and reprobed with 1B15 to control for loading.

sequence that matches seven of eight nucleotides of the CRE octamer motif present in cAMP-responsive genes, such as the rat somatostatin gene.[39] Forskolin, a diterpene that increases intracellular cAMP by stimulation of adenylate-cyclase activity, increases the fractional release of NT peptide and increases NT mRNA abundance at 1 hour after addition to BON cells. Conversely the addition of 5-HT, which we have shown decreases cAMP levels in BON,[21] decreases NT mRNA at 1 hour. At 3 hours after adding either FSK or 5-HT, NT mRNA levels had returned to control values. These findings suggest that the control of human NT gene expression and peptide secretion are mediated in part by activation of the cAMP signal-transduction pathway.

The rapid turnover in steady-state levels of NT mRNA found in this study suggests that the half-life of the NT mRNA transcript is relatively short. Similar transcript lability has been noted with certain proto-oncogenes (for example c-*myc* and c-*fos*) that are important in cellular proliferation,[40,41] and in other cAMP-regulated genes.[36,42]

We have found definite, albeit modest, cAMP-mediated effects on the regulation of NT expression in BON cells. Others[31,32] have reported little change in rat NT mRNA levels in PC12 cells after single addition of the inducing-agents FSK, dexamethasone, and nerve-growth factor; when these agents were added in combination, however, there was a significant synergistic induction in the expression of NT. This suggests that multiple environmental stimuli are necessary to produce a concerted transcriptional response of the NT gene. We currently are studying the effects of the addition of multiple inducing agents to BON cells to determine whether the transcriptional response of human NT can be enhanced to a degree similar to that found with the rat NT gene.

In conclusion we have identified a human carcinoid cell line that expresses the human NT gene and secretes NT peptide, both in the basal (uninduced) state and after induction with FSK. Our unique human carcinoid cell line, BON, will be a useful model to further define intracellular mechanisms involved in the transcriptional regulation and release of human NT.

References

1. Polak JM, Sullivan SN, Bloom SR, et al. Specific localization of neurotensin to the N cell in human intestine by radioimmunoassay and immunocytochemistry. Nature 1977; 270:183–184.
2. Helmstaedter V, Taugner CH, Feurle GE, Forssmann WG. Localization of neurotensin-immunoreactive cells in the small intestine of man and various mammals. Histochemistry 1977; 53:35–41.
3. Doyle H, Greeley GH Jr, Mate L, et al. Distribution of neurotensin in the canine gastrointestinal tract. Surgery 1985; 97:337–341.
4. Evers BM, Ishizuka J, Townsend CM Jr, et al. Posttranscriptional regulation of neurotensin in the gut. Surgery 1991; 110:247–252.
5. Thor K, Rosell S. Neurotensin increases colonic motility. Gastroenterology 1986; 90:27–31.
6. Sakamoto T, Newman J, Fujimura M, et al. Role of neurotensin in pancreatic secretion. Surgery 1984; 96:146–153.
7. Blackburn AM, Fletcher DR, Bloom SR, et al. Effect of neurotensin on gastric function in man. Lancet 1980; 1:987–989.
8. Andersson S, Rosell S, Hjelmquist U, et al. Inhibition of gastric and intestinal motor activity in dogs by (gln^4) neurotensin. Acta Physiol Scand 1977; 100:231–235.
9. Wood JG, Hoang HD, Bussjaeger LJ, Soloman TE. Neurotensin stimulates growth of small intestine in rats. Am J Physiol 1988; 255:G813–G817.
10. Feurle GE, Muller B, Rix E. Neurotensin induces hyperplasia of the pancreas and growth of the gastric antrum in rats. Gut 1987; 28: S1,19–23.
11. Wood JG, Hoang HD, Bussjaeger LJ, Solomon TE. Effect of neurotensin on pancreatic and gastric secretion and growth in rats. Pancreas 1988; 3:332–339.
12. Evers BM, Mehrabian H, Townsend CM Jr, et al. Neurotensin prevents small bowel mucosal atrophy produced by elemental diet. Surg Forum 1989; 40:22–24.
13. Evers BM, Izukura M, Townsend CM Jr, et al. Differential effects of gut hormones on pancreatic and intestinal growth during administration of an elemental diet. Ann Surg 1990; 211:630–638.
14. Moody TW, Carney DN, Korman LY, et al. Neurotensin is produced by and secreted from classic small cell lung cancer cells. Life Sci 1985; 36:1727–1732.
15. Staley J, Fiskum G, Davis TP, Moody TW. Neurotensin elevates cytosolic calcium in small cell lung cancer cells. Peptides 1989; 10:1217–1221.
16. Davis TP, Burgess HS, Crowell S, et al. β-Endorphin and neurotensin stimulate *in vitro* clonal growth of human SCLC cells. Eur J Pharmacol 1989; 161:283–285.
17. Evers BM, Townsend CM Jr, Upp JR, et al. Establishment and characterization of a human carcinoid in nude mice and effect of various agents on tumor growth. Gastroenterology 1991; 101: 303–311.
18. Lawrence JP, Haber B, Ishizuka J, Thompson JC. The effect of various gastrin forms on secretion of a carcinoid tumor *in vitro*. Gastroenterology 1989; 96:A291.
19. Jeng Y-J, Townsend CM Jr, Nagasawa S, et al. Regulation of pancreastatin release from a human pancreatic carcinoid cell line *in vitro*. Endocrinology 1991; 128:220–225.
20. Chuo S, Townsend CM Jr, Farrar S, et al. Coordinate pancreastatin and chromogranin A secretion from a human pancreatic carcinoid: evidence of chromogranin processing to pancreastatin. Gastroenterology 1990; 98:A653.
21. Ishizuka J, Beauchamp RD, Greeley GH Jr, et al. 5-Hydroxytryptamine is an autocrine factor for human pancreatic carcinoid. Surg Forum 1990; 41:182–184.
22. Allen E, Evers BM, Townsend CM Jr, et al. Somatostatin analog (201–995) and α-difluoromethylornithine (DFMO) inhibit growth of human carcinoid tumor. Surg Forum 1989; 40:415–417.
23. Evers BM, Hurlbut SC, Tyring SK, et al. Novel therapy for the treatment of human carcinoid. Ann Surg 1991; 213:411–416.
24. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162:156–159.
25. Dobner PR, Barber DL, Villa-Komaroff L, McKiernan C. Cloning and sequence analysis of cDNA for the canine neurotensin/neuromedin N precursor. Proc Natl Acad Sci USA 1987; 84:3516–3520.
26. Danielson PE, Forss-Petter S, Brow MA, et al. p1B15: a cDNA clone of the rat mRNA encoding cyclophilin. DNA 1988; 7:261–267.
27. Kislauskis E, Bullock B, McNeil S, Dobner PR. The rat gene encoding neurotensin and neuromedin N structure, tissue-specific expression, and evolution of exon sequences. J Biol Chem 1988; 263: 4963–4968.
28. Kapuscinski M, Shulkes A, Read D, Hardy KJ. Expression of neurotensin in endocrine tumors. J Clin Endocrinol Metab 1990; 70:100–106.
29. Carraway RE, Mitra SP, Feurle GE, Hacki WH. Presence of neurotensin and neuromedin-N within a common precursor from a human pancreatic neuroendocrine tumor. J Clin Endocrinol Metab 1988; 66:1323–1328.
30. Greene LA, Tischler AS. Establishment of a noradrenergic clonal

line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. Proc Natl Acad Sci USA 1976; 73:2424–2428.

31. Dobner PR, Tischler AS, Lee YC, et al. Lithium dramatically potentiates neurotensin/neuromedin N gene expression. J Biol Chem 1988; 263:13983–13986.
32. Kislauskis E, Dobner PR. Mutually dependent response elements in the *cis*-regulatory region of the neurotensin/neuromedin N gene integrate environmental stimuli in PC12 cells. Neuron 1990; 4:783–795.
33. Zeytin FN, DeLellis R. The neuropeptide-synthesizing rat 44-2C cell line: morphological characterization and regulation of peptide synthesis, secretion, adenosine 3′, 5′-monophosphate efflux and adenylate cyclase activation. Endocrinology 1987; 121:352–360.
34. Zeytin FN, Rusk SF, Baird A, et al. Induction of c-*fos*, calcitonin gene expression, and acidic fibroblast growth factor production in a multipeptide-secreting neuroendocrine cell line. Endocrinology 1988; 122:1114–1120.
35. Habener JF. Cyclic AMP response element binding proteins: a cornucopia of transcription factors. Mol Endocrinol 1990; 4:1087–1094.
36. Roesler WJ, Vandenbark GR, Hanson RW. Cyclic AMP and the induction of eukaryotic gene transcription. J Biol Chem 1988; 263:9063–9066.
37. Darnell JE Jr. Variety in the level of gene control in eukaryotic cells. Nature 1982; 297:365–371.
38. Cantley LC, Auger KR, Carpenter C, et al. Oncogenes and signal transduction. Cell 1991; 64:281–302.
39. Montminy MR, Sevarino KA, Wagner JA, et al. Identification of a cyclic-AMP-responsive element within the rat somatostatin gene. Proc Natl Acad Sci USA 1986; 83:6682–6686.
40. Treisman R. Transient accumulation of c-*fos* RNA following serum stimulation requires a conserved 5′ element and c-*fos* 3′ sequences. Cell 1985; 42:889–902.
41. Beimling P, Benter T, Sander T, Moilling K. Isolation and characterization of the human cellular *myc* gene product. Biochemistry 1985; 24:6349–6355.
42. Cimbala MA, Lamers WH, Nelson K, et al. Rapid changes in the concentration of phosphoenolpyruvate carboxykinase mRNA in rat liver and kidney. J Biol Chem 1982; 257:7629–7636.

DISCUSSION

DR. HAILE T. DEBAS (San Francisco, California): I believe Drs. Evers, Townsend, and Thompson asked me to discuss this paper in the hope, rather than the belief, they would nudge me into the new era of molecular biology. I have to assure them that they failed in that effort.

This group is to be congratulated for its success in developing this unique human carcinoid cell line. Having shown that there is cyclic and mediated transcription of regulation of neurotensin, they are now poised to ask the important questions of what happens proximal and distal to the forskolin step. Specifically is the initiation of the intracellular process purely dependent on adenylate cyclase activation or are other regulatory membrane proteins involved?

And more importantly, how is the signal transferred to the nucleus for transcription to occur? If there is autocrine growth regulation by neurotensin, is this mediated by a different pathway; specifically are proto-oncogenes involved?

I have two questions for Dr. Evers. First you have shown increased message in response to forskolin, but the important issue is showing translation. Specifically how well did the increase in neurotensin messenger RNA correlate, both temporarily and in the dose-response relationship, to the secretion of neurotensin as measured by RIA?

Second you have shown that neurotensin is transcribed in two messenger RNAs. Do you think this is specific splicing that might have functional significance? If so could you hypothesize on the significance of the two messenger RNAs?

Thank you.

DR. DANA K. ANDERSEN (Chicago, Illinois): This laboratory has established a tradition at this meeting of providing state-of-the-art information on the biology and surgical significance of gastrointestinal endocrinology, and this paper certainly extends this tradition.

Perhaps the real significance of this work is the totality of the accomplishment. A portion of a metastatic tumor was transferred to a stable *in vivo* system, which allows the examination of mechanisms responsible for tumor function. The expression of specific genes, such as the neurotensin-neuromedin-C gene, has been quantified, and specific agents that promote or suppress the expression of these genes can be identified. This is an outstanding accomplishment.

Gene expression, or the transcription of mRNA from the gene, is a vital step in our understanding of the function of the tumor cell because the mere measurement of the final secretory product, in this case neurotensin, fails to provide a complete picture of the function of the cell. For example this study shows that serotonin suppresses the expression of the neurotensin gene. But this fact would go unnoticed if we relied on the measurement of neurotensin alone, as little neurotensin secretion was observed under basal circumstances.

That being the case, my first question is whether the authors could detect a reduction in cellular neurotensin content coincident with the suppression of neurotensin mRNA, and if not, whether they can provide any corroborative evidence that this suppression has any functional outcome on the secretory or growth activities of this tumor?

Because the tumor is a foregut carcinoid that produces serotinin, my second question is whether the tumor tonically suppresses neurotensin gene expression because of its own endogenous serotonin synthesis? Are the levels of serotonin administered to the cell culture similar to the levels of serotonin one might expect in the intracellular or extracellular compartments of the tumor? Does ongoing synthesis and secretion of serotonin by the tumor result in permanent suppression of neurotensin gene expression and synthesis of neurotensin by the tumor? If so one wonders why or how neurotensin is produced at all by this tumor.

This study corroborates the finding that expression of the neurotensin gene is related to a cyclic AMP–dependent element of the gene itself. One wonders, however, whether the expression of a whole host of genes is not stimulated by raising endogenous cyclic AMP levels by the addition of forskolin. Is there some specificity for synthetic processes related to secretory as opposed to growth responses of the tumor?

Finally although the data show recovery of the mRNA transcription stimulated by forskolin back to basal levels, and the authors conclude that induced transcription therefore is time limited, I would ask also whether this recovery might not simply be due to decreasing availability of cyclic AMP produced by forskolin?

DR. JOHN NIEDERHUBER (Baltimore, Maryland): The task before this particular investigator and his group is to use a specific cell line to look at signal transduction pathways and to ask specific questions about a given gene that they have identified as potentially involved in this process. Their goal is to study the expression of this gene and to characterize the resultant peptide product of the gene.

I think you have gathered from the comments of the previous two discussants that these are complex and difficult questions.

The obvious cautions with such a model, of course, need to be noted. Their model involves the use of a transformed cell line. As the investigators learn more about the expression of their specific gene, they will have to make constant comparisons between the abnormal cell and appropriate normal cells. They will need to prove that BON cells use the same mechanism of triggering NT gene expression as are used by normal cells and that the promoter region of the BON NT gene is unaltered. This laboratory group will obviously do that.

A question I would like to raise relates to forskolin and serotonin used in their experiments. These agonists tend to be fairly pleomorphic in their effects on the cell, a fact alluded to by the other discussants. Perhaps Dr. Evers could comment on why these two agents were selected instead of perhaps other agonists; for example dibuteryl cyclic AMP or phosphodiesterase inhibitors such as theophylin—reagents that have a more direct effect on cyclic AMP.

Finally the authors have implied that because the rat NT-gene promoter contains a DNA sequence that matches seven of eight nucleotides known to be present in the CRE octamer control region of other cAMP-responsive genes, the NT gene in BON cells is likely to be mediated by a cAMP pathway. Although this may prove to be correct, it ultimately will depend on the cloning and sequencing of the promoter region of the BON-NT gene for more direct comparison and for the needed studies of NT-gene expression.

DR. M. DAVID TILLSON, III (New York, New York): You made some remarks in your introduction that I would like to ask you a question about, because I wonder if perhaps the loop can be closed now on some questions that I was interested in several years ago on the mechanisms related to adaptation of the gut and regulation of intestinal mass. I wonder if neurotensin is detectable in serum, and if addition of neurotensin stimulates intestinal growth in normal mucosa, even when it is not previously atrophied by starvation, and whether or not it is elevated after small bowel resection, and perhaps what would be most interesting to me of all, whether neurotensin might be the mechanism that we observed in some experiments years ago on the effect of small intestinal resection stimulating carcinogenesis in the rat colon in response to dimethylhydrazine?

I realize your paper was on a different subject, but is there a physiologic role for this hormone, is it a candidate for the regulation of intestinal mass, and does it perhaps have implications in terms of preparing the colon as a fertile soil for carcinogens?

DR. STANLEY R. FRIESEN (Kansas City, Kansas): There have been several interesting papers this afternoon that have dealt with an increasing spectrum of physiologic and pharmacologic actions of humoral substances, such as growth factors and neuropeptides. There are newer neuropeptides being described that have neurotransmitter or neuroendocrine actions.

Also it is increasingly apparent that these regulatory and trophic peptides reside not only in mucosal endocrine cells, but also in neural cells, such as ganglion cells of the gut and pancreas. When neural cells become neoplastic, they may liberate neuropeptides in excess. Examples include carcinoid-appearing tumors of the duodenal submucosa or of the pancreas; or, as in this interesting paper, a pancreatic carcinoid cell line that secretes neurotensin.

Historically it was Masson who described neural hyperplasia associated with carcinoid tumors of the gut. And Langerhans, a little over a hundred years ago, described a third group of cells in the pancreas, the neural ganglion cells between the acinar and the islet cells. It may be that neural cells constitute the cells of origin of gastrinomas that secrete the neuropeptide gastrin.

DR. B. MARK EVERS (Closing discussion): Dr. Debas, you asked about neurotensin mRNA levels in correlation with actual neurotensin protein levels. We found similar increases in both the message for neurotensin and neurotensin protein levels in BON cells after forskolin stimulation.

You next asked about possible functions of the two neurotensin transcripts. These two mRNA species result from the use of two different polyadenylation addition signals, and the two mRNAs differ in the extent of their 3′ untranslated regions. It is interesting that the abundance of these two transcripts is different, depending on the tissue sampled. For example the gut has predominantly the smaller of the two mRNAs, whereas the brain has nearly equal amounts of both transcripts. We cannot say, however, whether this has any particular significance.

Dr. Andersen, you asked whether serotonin (5-HT) decreases neurotensin release. Ishizuka and colleagues, from our laboratory, have shown that 5-HT produces a dose-dependent decrease of cyclic AMP levels in BON cells, and, in addition, 5-HT decreases neurotensin release.

You also asked whether 5-HT may suppress endogenous neurotensin release given the fact that BON cells produce 5-HT. We suspect that there is some degree of basal suppression of neurotensin. In preliminary studies we have used a 5-HT receptor antagonist, LM-21009, which blocks $5\text{-HT}_{1A/1B}$ receptors. When LM-21009 is administered to BON cells, neurotensin release is increased. We are currently evaluating whether this increase in neurotensin release is correlated with an increase in neurotensin gene expression.

We did not measure cyclic AMP levels over a time course after administering forskolin. We postulate that the rapid changes in neurotensin mRNA levels are secondary to a relatively short half-life of the neurotensin transcript.

Dr. Niederhuber, we are currently evaluating the effects of other agents that increase intracellular cyclic AMP levels on neurotensin expression in BON. We used forskolin initially because it can directly activate adenylate cyclase and is quite stable.

Dr. Tilson, we have preliminary findings to suggest that neurotensin may play a role in the adaptive response of the remaining gut after small bowel resection. We have found that neurotensin mRNA levels are increased as early as 3 hours in the remaining small bowel after a 70% resection.

You also asked whether neurotensin could affect the growth of normal small bowel mucosa. It has been shown by several groups, including our own laboratory, that neurotensin can stimulate growth of normal small bowel mucosa and pancreas.

Dr. Friesen, you asked whether neurotensin could affect the growth of BON cells. Neurotensin has no effect on the growth of BON; however we have found that BON has no receptor for neurotensin.

Lung and Heart–Lung Transplantation

Evolution and New Applications

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Heart–lung transplantation (HLT) and lung transplantation (LT) are effective treatment modalities for patients with advanced pulmonary parenchymal or vascular disease. Lung transplantation offers potential advantages over HLT, including reduced pretransplant waiting time and improved efficiency of organ utilization, and is currently being offered to patients formerly treated by HLT. To explore the relative merits of these procedures, the authors examined the results in 44 procedures (23 HLT and 21 LT) in 42 patients transplanted at their institution. Heart–lung transplant recipients included 20 adults and three children (ages 5, 5 and 3). Most HLT patients had primary pulmonary hypertension (PPH) (n = 9) or Eisenmenger's syndrome (ES) (n = 8). Twenty-two of twenty-three patients have been long-term survivors (mean follow-up = 17.8 months, Kaplan-Meier survival at 12 months = 85%). Obliterative bronchiolitis (OB) has occurred in five patients (22%), and all have died. Of 21 LTs in 19 patients, nine had obstructive and eight had restrictive lung diseases. Three single-LT (SLT) patients had PPH, and one had ES secondary to a ventricular septal defect. Mean pulmonary artery pressures fell from 55 ± 6 mm Hg before SLT to 21 ± 3 mm Hg after SLT; $p < 0.001$. Three pediatric patients (ages 4, 10, 17, and 17[re-transplant]) have undergone four SLTs. With mean follow-up of 6.4 months, LT patients have survival at 12 months of 80% (Kaplan-Meier). Lung transplant patients wait a far shorter time for their transplant than do HLT patients (166 *vs.* 384 days, $p < 0.03$). Three patients (19%) have evidence of OB after SLT, with one death. By virtue of equal intermediate-term outcomes, shorter waiting times, and better use of donor organs in comparison with HLT, LT should be offered whenever possible to patients with end-stage pulmonary parenchymal or vascular disease. The authors' pediatric LT and HLT experience (7 treatments in 6 patients) is the largest reported to date and demonstrates the utility of these procedures in this group. Chronic rejection (OB) remains the greatest impediment to long-term survival in both LT and HLT pts.

AFTER THE PIONEERING work of Reitz et al.,[1] who demonstrated the feasibility of transplanting pulmonary tissue with the first successful heart–lung allografts, and Cooper et al.,[2] who first successfully transplanted a single lung, recent years have witnessed a rapid evolution in the approach to the patient with end-stage lung disease. Primarily because of the severe limitation in available donor organs, many patients who formerly would have been offered heart–lung transplantation are now receiving lung transplants. In this report, we describe our experience with heart–lung and lung transplantation and offer our views regarding the relative merits of, and indications for, these two procedures.

Materials and Methods

Recipient Selection

Only patients with end-stage pulmonary or cardiopulmonary disease are considered for transplantation. All patients are disabled and most require supplemental oxygen therapy. There must be no other medical or surgical options available that are deemed likely to afford any substantial benefit within a reasonable degree of medical certainty. All individuals selected are deemed to be experiencing the last 24 to 36 months of their natural lives. Patients are screened for the presence of other disorders that might have an impact on survival independent of their pulmonary or cardiopulmonary disease. Psychosocial suitability and compliance also are analyzed before placement on the waiting list.

Donor Selection

Donors in all cases were multiple-organ donors. Donor selection criteria are listed in Table 1. Size matching is relatively more important in cases of heart–lung or bilateral lung transplantation. In these cases it is imperative not to oversize the donor lungs. In single-lung transplants,

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a greater disparity in size between donor and recipient may be tolerated. A lung from an individual either larger or smaller than the recipient may be accommodated because of the ability of the mediastinum to shift and the contralateral lung to expand or be compressed, such that space problems have not been prevalent despite large differences in lung volumes between donor and recipient. Pulmonary function must be well maintained. Arterial PaO_2 of 100 mm Hg or greater on a fraction of inspired oxygen (FIO_2) of 0.4 must be demonstrated. In addition lung compliance must be normal (peak airway pressure of no greater than 30 mm Hg with a normal tidal volume). The chest radiograph should be clear, and sampled pulmonary secretions should demonstrate no gram-negative or fungal organisms. Because of the potential occurrence of graft-*versus*-host disease in the form of hemolytic anemia with its attendant complications when ABO compatibility, but not identity, is observed, we currently require ABO identity between donor and recipient.

Operative Strategies

Heart–Lung Transplantation. All heart–lung transplantation (HLT) procedures are performed with cardiopulmonary bypass. Meticulous attention to posterior mediastinal homostasis must be observed. The heart is removed and the phrenic nerves are isolated on generous pedicles of pericardium. Subsequently the lungs are individually removed by dividing the inferior pulmonary ligaments and transecting the pulmonary hilar structures with a TA-90 surgical stapling device. This facilitates the removal of the lungs and improves hemostasis. Sufficient posterior left atrium is then removed to allow unimpeded passage of the donor right lung posterior to the right atrium and into the right pleural space. The donor heart–lung bloc then is placed into the recipient chest and the tracheal anastomosis is performed. Continuous polypropylene is employed in the adult, and interrupted polypropylene in the pediatric age group, to allow growth to occur at the anastomotic site. Donor and recipient aorta and right atrium then are anastomosed, completing the implant.

TABLE 1. *Donor Selection Criteria*

Criteria
Immunologic
ABO identical
Crossmatch required in sensitized patients
Pulmonary function
Chest radiograph clear
paO_2 100 mmHg or > on FiO_2 0.4
Lung complicance normal
Peak airway pressure 30 mmHg or less with normal tidal volume
Microbiologic
No obvious pulmonary sepsis
Pulmonary secretions
Absence of gram-negative or fungal organisms
Size match
Heart–lung or bilateral lung transplant
Donor lung volume same or less than recipient
Single lung transplant
Donor lung volume less critical
May be larger than recipient

ABO, ABO blood group system.

Lung Transplantation. Single-lung transplants (SLTs) are performed through an anterolateral thoracotomy, employing modifications of the technique described in animals by Metras in 1950[3] and by Hardin and Kittle in 1954.[4] Patients are intubated with a double-lumen endotracheal tube to allow the lung being replaced to be deflated. This allows the hilar structures to be mobilized and divided. We have found it advantageous to occlude the ipsilateral pulmonary artery with a tourniquet soon after entering the chest and deflating the lung. This maneuver prevents shunting of blood through an unventilated lung, and resultant hypoxemia. If the patient develops unacceptable levels of hypoxemia or hypercarbia, the lung can be reperfused and ventilated while cardiopulmonary bypass is instituted by the femoral approach or through the chest. All patients with pulmonary hypertension, either primary pulmonary hypertension (PPH) or Eisenmenger's syndrome (ES), who undergo SLT require the institution of cardiopulmonary bypass. Figures 1 through 3 depict the technique of SLT. In Figure 1, the diseased recipient lung has been removed. The bronchial anastomosis has been accomplished with interrupted nonabsorbable suture material. Vascular clamps are in position on the pulmonary artery and across the base of the pulmonary veins. Figure 2 shows the omental pedicle being wrapped around the bronchial anastomosis for protection, and to provide an exogenous blood supply. In Figure 3, the pulmonary artery anastomosis has been completed, and the anastomosis between donor and recipient left atrial cuffs is being accomplished. This anastomosis completes the implant procedure. The lung is flushed antegrade and retrograde with blood, and the clamps are released, restoring perfusion to the lung. When hemostasis at the pulmonary hilum is secure, ventilation of the graft can be initiated.

Bilateral Single-lung Transplantation. The technique of *en bloc* double-lung transplantation with tracheal and posterior left atrial anastomoses as reported by the Toronto group is of historic interest only because of the reported high incidence of airway complications attending its use.[5,6] This procedure has been modified by the Washington University group of Pasque et al.[7] to a method of bilateral SLTs with individual bronchial anastomoses. This procedure can be performed without the routine necessity of cardiopulmonary bypass in certain cases. With the double-lumen endotracheal tube and careful monitoring of arterial blood gases, it is possible to alternately

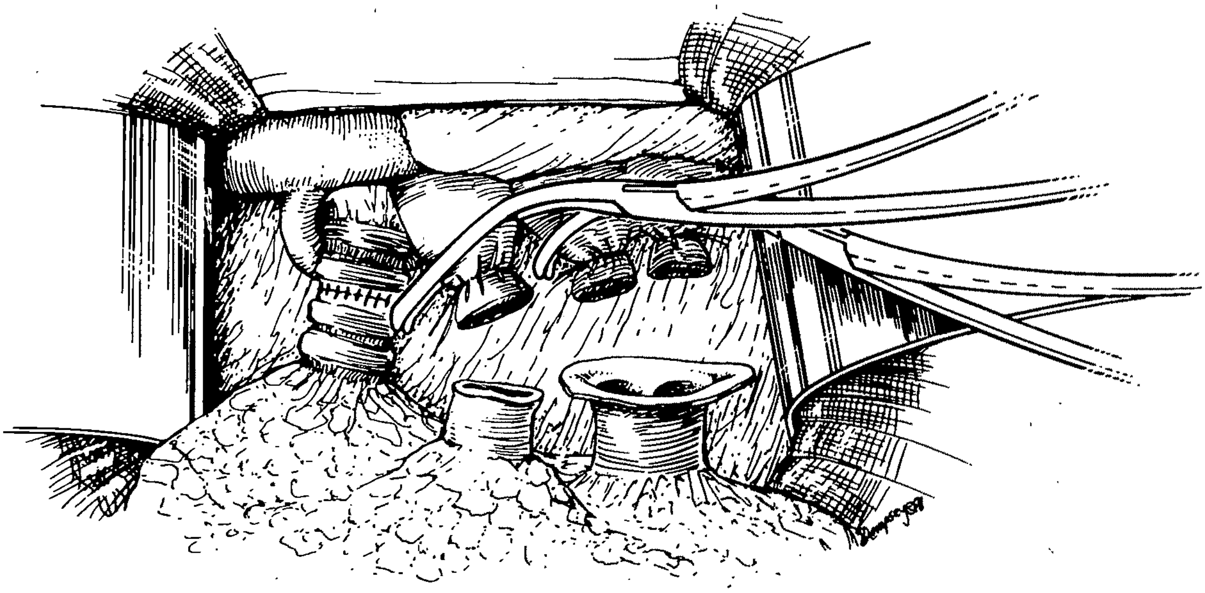

FIG. 1. Technique of right single lung transplant. Bronchial anastomosis has already been completed.

deflate the diseased lungs to mobilize the hilar structures. One allograft lung then can be implanted and the patient supported with this graft during implantation of the second, contralateral graft. This procedure is greatly facilitated by the use of the bilateral transverse thoracotomy, crossing the sternum at the level of the fourth intercostal space. The exposure is excellent, and the avoidance of bypass can greatly decrease operative hemorrhage.

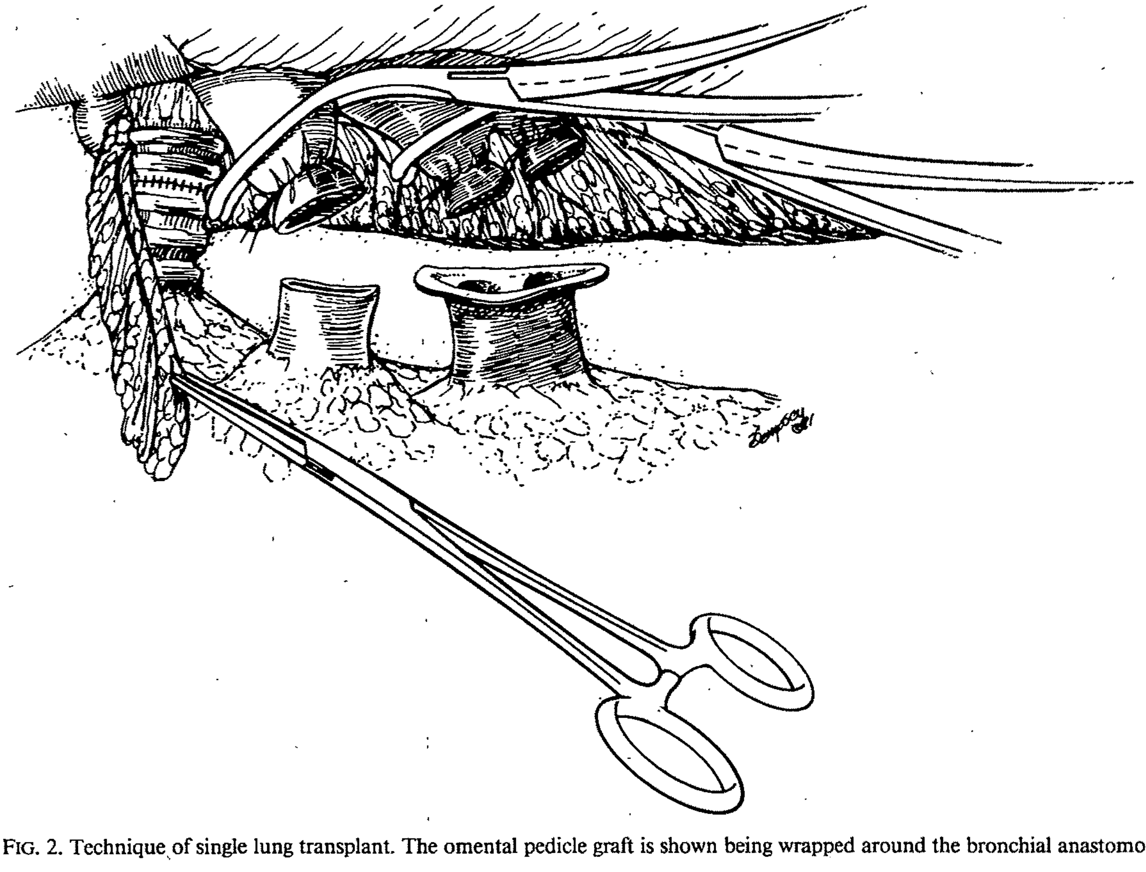

FIG. 2. Technique of single lung transplant. The omental pedicle graft is shown being wrapped around the bronchial anastomosis.

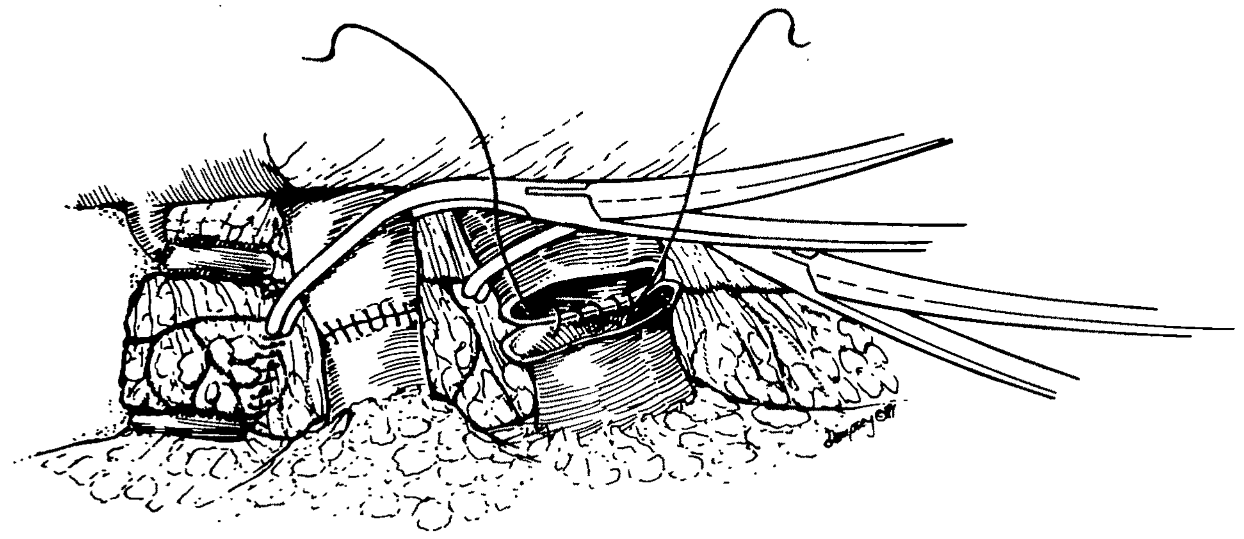

FIG. 3. Technique of single lung transplant. The completed pulmonary artery anastomosis is shown, and the left atrial anastomosis is being performed. This anastomosis completes the implant procedure.

Immunosuppression

All recipients receive immunosuppression according to the so-called "triple-therapy" protocol introduced at the University of Minnesota in 1983 for cardiac transplantation.[8] Table 2 outlines the details of this regimen. Cyclosporine (CSA) is initiated preoperatively in a small dose (4–8 mg/kg), depending on renal function. Postoperatively CSA is administered as a low-dose continuous infusion (2–3 mg/hr). In addition, CSA is administered orally in a dose of 4 to 6 mg/kg/day in two divided doses 12 hours apart. Azathioprine is administered preoperatively in a dose of 2 to 3 mg/kg and postoperatively is targeted to a white blood cell count of 4000 to 5000 cells/mm^3. Methylprednisolone is administered intraoperatively in a dose of 500 mg and postoperatively 125 mg every 8 hours for three doses. Steroids then are withheld for 10 to 14 days to promote healing of the airway anastomosis. At around day 10 to 14, prednisone is initiated at 0.5 mg/kg/day. A portion of the recipients have received antilymphocyte therapy in the form of Minnesota anti-lymphocyte globulin for 3 to 5 days after transplant. This allows the use of lower CSA doses in the interest of preserving optimal renal function during the critical first several days.

TABLE 2. *Immunosuppression for Lung, Heart-Lung Recipients*

Treatment

- Preoperative
  - CSA 4–6 mg/kg orally depending on renal function
  - AZA 2–3 mg/kg orally
- Intraoperative
  - MP 500 mg I.V. at time of reperfusion
- Postoperative
  - CSA
    - Oral (NG) 2–4 mg/kg/day in two divided doses
    - I.V. 2–3 mg/hr as continuous infusion
  - AZA 2–3 mg/kg/day orally (NG)
    - Decrease for WBC < 5000/mm^3
  - MP 125 mg I.V. every 8 hours for three doses
  - Minnesota anti-lymphoblast globulin (MALG) 15 mg/kg I.V. every day for 3 to 5 days
  - Prednisone beginning day 10–14 0.5 mg/kg/day
- Maintenance
  - CSA 5–6 mg/kg/day in two doses
  - AZA 1.5–2.5 mg/kg/day
  - Prednisone taper to 0.1 mg/kg/day by 3 to 6 months

CSA, cyclosporine; AZA, azathioprine; MP, methylprednisolone; NG, nasogastric.

Infection Prophylaxis

All patients receive perioperative vancomycin for the 24 hours surrounding surgery and cefamandol until all drainage catheters and monitoring lines are removed. Recipients who are cytomegalovirus (CMV) seronegative before transplant receive exclusively CMV-negative blood and blood products. All patients receive high-dose acyclovir (3200 mg/day) and mycostatin for 90 days after transplant, and trimethoprim-sulfamethoxazole indefinitely. Donor and recipient airways are cultured at the time of surgery to facilitate a more rational selection of antibiotic therapy in the early post-transplant period. Every attempt is made to treat only identified infections and to avoid the indiscriminate use of antibiotics, lest fungal or resistant bacterial overgrowth develop.

Postoperative Surveillance

Patients are assessed carefully for signs of infection and rejection in the post-transplant period. Fiberoptic bronchoscopy (FOB) is employed liberally in this effort. Bronchoalveolar lavage (BAL) has proven of great utility in the diagnosis of infection and transbronchial biopsy (TBB) in the diagnosis and monitoring of rejection episodes. Fiberoptic bronchoscopy is performed generally within the first 24 hours of surgery to assess the airway anastomosis, and thereafter is indicated for any significant change in the patient's clinical status, chest radiograph, or arterial blood gases. Routine FOB with BAL and TBB is performed on a protocol basis every 7 to 10 days during the transplant hospitalization, and subsequently at gradually increasing intervals after discharge from the hospital. Long-term patients undergo FOB with BAL and TBB every 3 to 6 months.

Results

Indications

Tables 3 and 4 list the indications for HLT and lung transplantation (LT), respectively. Most HLTs were performed for pulmonary vascular disease, either PPH (9 patients) or secondary to a congenital heart defect (ES) (8 patients). Three HLTs were performed for obstructive lung disease (alpha-1 antitrypsin deficiency [A1ATD]). One patient each had HLT for cystic fibrosis (age 5), lymphocytic interstitial pneumonitis (LIP) (age 6), and obliterative bronchiolitis (OB) from a viral infection (age 3). This child had been ventilator dependent since birth because of infant respiratory distress syndrome and bronchopulmonary dysplasia, when he developed superimposed OB.

Among patients undergoing LT, nine had obstructive airways disease (7 A1ATD, 2 COPD). Three had PPH, one in association with Histiocytosis X. Three had idiopathic pulmonary fibrosis, and one each had lymphangioleiomyomatosis, OB after previous HLT, ES from a ventricular septal defect, cystic fibrosis, and LIP. There have been two retransplants; one, a SLT in a 10-year-old girl 2 years status post HLT who developed OB, and the other in a 17-year-old girl 1 month after unsuccessful SLT for LIP. The original allografted lung demonstrated refractory rejection as well as OB.

TABLE 3. *Indications for Heart–Lung Transplant*

Underlying Disease	No. of Transplants
Primary pulmonary hypertension	9
Eisenmenger's syndrome	8
Emphysema due to alpha-1 antitrypsin deficiency	3
Cystic fibrosis	1
Lymphocytic interstitial pneumonitis	1
Bronchopulmonary dysplasia with obliterative bronchiolitis	1
Total	23

TABLE 4. *Indications for Lung Transplant*

Disease	No. of Patients	Transplant Procedure
Alpha-1 antitrypsin deficiency	6	SLT
	1	DLT
	1	BSLT
Primary pulmonary hypertension	3	SLT
VSD, pulmonary hypertension	1	VSD closure, SLT
Emphysema	1	SLT
Lymphangioleiomyomatosis	1	SLT
Lymphocytic intersititial pneumonitis	1	SLT
Idiopathic pulmonary fibrosis	3	SLT
Obliterative bronchiolitis	1	SLT
Refractory rejection	1	SLT
Cystic fibrosis	1	BSLT
Total	21	

SLT, single lung transplant; DLT, double lung transplant; BSLT, bilateral single lung transplant; VSD, ventricular septal defect.

Waiting Times

Time on the waiting list was compared between patients undergoing HLT and those undergoing SLT as well as all lung-only transplants. As shown in Figure 4, the waiting time for SLT was 150 ± 42 days, compared with 384 ± 88 days for HLT ($p < 0.02$). Waiting time for all lung-only transplants was 166 ± 39 days ($p < 0.03$, compared with HLT).

Operative Procedures

Table 5 lists the operative procedures that have been performed in the patients in our series. There have been 23 HLTs, and in three instances, the recipient's heart has been transplanted into another individual (the so-called "dominoe" living donor procedure, as reported by Baumgartner et al.[9]) Seventeen patients have received 18 SLTs. Three patients have undergone bilateral lung replacement, one with an *en bloc* double-lung, and two with bilateral single-lung transplants.

Operative Survival

There has been one death within 30 days of transplant among HLT patients (4%). This death was due to uncontrollable hemorrhage in a 45-year-old man with a patent ductus arteriosus. All other HLT patients have been long-term survivors. Among LT patients, there have been four deaths within 30 days of transplant (19%). One death was due to cerebral hypoxemia from an intraoperative cardiac arrest. Another was due to poor pulmonary preservation (the first LT patient in our series). One patient had a massive pulmonary embolus to the transplant lung on postoperative day 4 and subsequently died. The final death was in the patient with ES from a ventricular septal defect (VSD). He had unsuspected right ventricular outflow tract

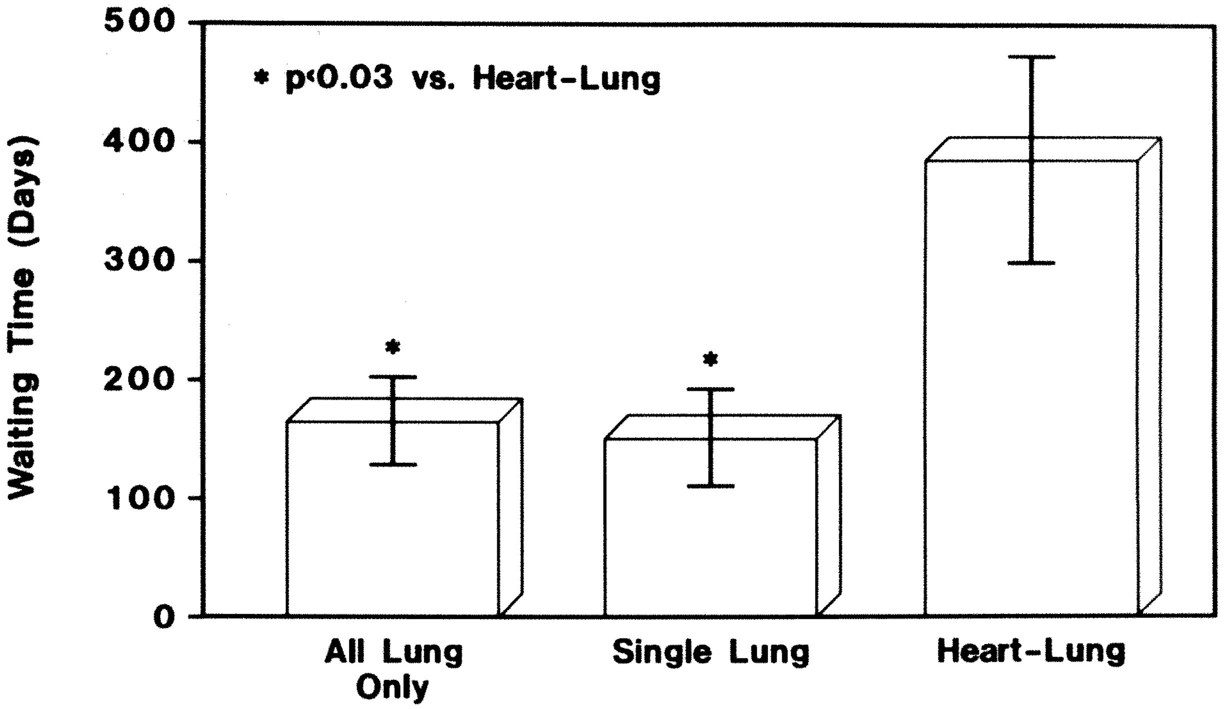

FIG. 4. Comparison of waiting times (mean ± SE) for all lung-only, single lung, and heart–lung transplant recipients.

obstruction, which was unmasked by SLT and closure of his VSD. He died because of low cardiac output from this right-sided obstructive lesion.

Pediatric Lung and Heart–Lung Transplantation

Six children have undergone a total of eight HLT and SLT procedures. These children ranged in age from 3 years to 17 years (mean, 7.3 years). Table 6 lists the diagnosis, patient age, and procedure. All procedures were performed on cardiopulmonary bypass. There has been one death. This occurred in a 10 year-old who had HLT for PPH at age 8. She developed OB and subsequently underwent SLT. She sustained an intraoperative cardiac arrest before the institution of cardiopulmonary bypass and died 5 days postoperatively of cerebral hypoxia. The other five patients are alive at 4 to 22 months after transplant. One child, a 4 year-old SLT for PPH, is awaiting retransplant for OB. Another, a 5 year-old HLT for lymphocytic interstitial pneumonitis, developed a lymphoproliferative disorder in the allograft lung. This has responded to gamma-globulin and alpha-interferon therapy. In addition, this child has evidence of recurrence of his original disease in his transplanted lungs. There has been one successful retransplant in a 17-year-old SLT recipient who developed refractory rejection and OB in her first allograft at 1 month after her first transplant. Three of the six patients are well, active, and not requiring supplemental oxygen.

TABLE 5. *Operative Procedures*

Procedure	No.
Heart–lung trasnplant—three dominoe procedures	23
Single lung transplant—one retransplant at 1 mo	18
Double lung transplant	1
Bilateral single lung transplant	2
Total	44

TABLE 6. *Pediatric Lung, Heart–Lung Transplant*

Diagnosis	Age (yr)	Procedure	Status
PPH	8	HLT*	OB at 18 mo, retransplant at 2 yr
PPH	4	SLT†	OB at 3 mo
Cystic fibrosis	5	HLT	A&W
LIP	5	HLT	Lymphoma at 3 mo, recurrent LIP at 1 yr
LIP	17	SLT	Refractory rejection, retransplant at 1 mo
OB	3	HLT	A&W, stenosis at tracheal anastomosis
OB (patient 1 above)	10	SLT	Died perioperatively
Allograft rejection (patient 5 above)	17	SLT, repeat	A&W 15 mo after second transplant

PPH, primary pulmonary hypertension; HLT, heart–lung transplant; OB, obliterative bronchiolitis; SLT, single lung transplant; A&W, alive and well; LIP, lymphocytic interstitial pneumonitis.

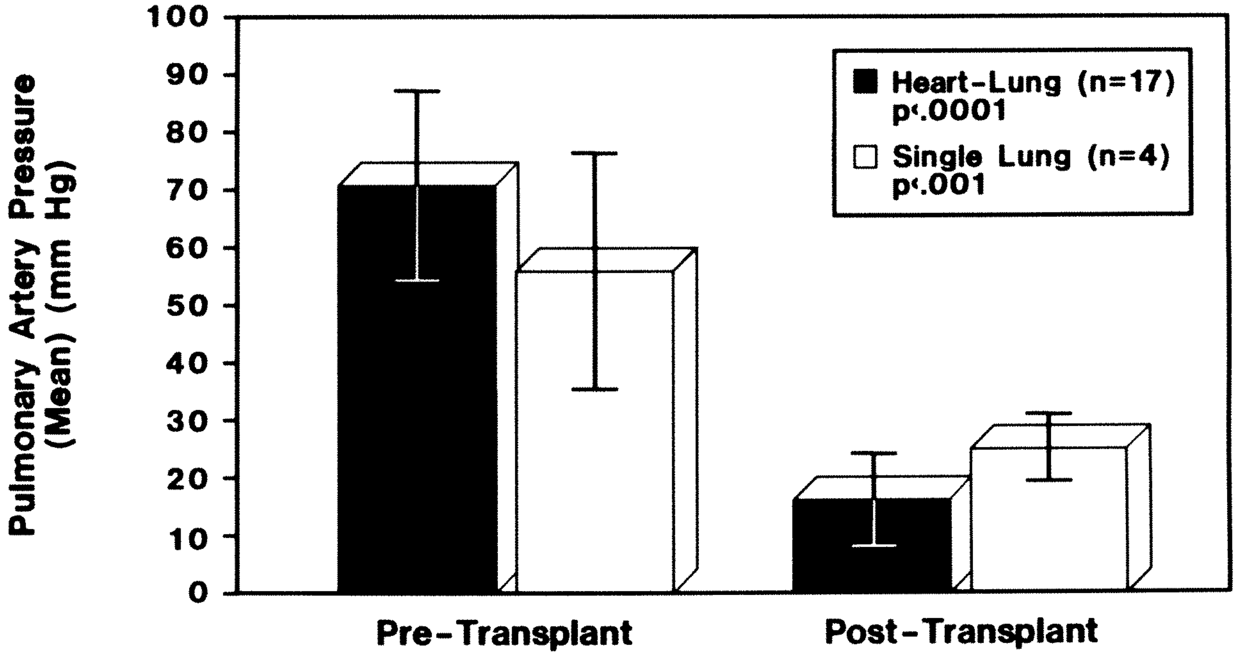

FIG. 5. Pulmonary artery pressures before and after heart-lung or single lung transplant for primary or secondary pulmonary hypertension.

Pulmonary Artery Pressures

There have been 17 HLTs and 4 SLTs performed for pulmonary hypertension, either primary (PPH) or secondary (ES). Figure 5 depicts the relative efficacy of these two procedures in lowering the mean pulmonary artery pressure (mPAP) after surgery. In the HLT patients, preoperative mPAP was 70 ± 17 mm Hg, and postoperative mPAP was 18 ± 9 mm Hg ($p < 0.0001$). In SLT recipients, preoperative mPAP was 57 ± 21 mm Hg and came down to 25 ± 5 mm Hg postoperatively ($p < 0.001$). Thus both procedures are capable of effecting a highly significant

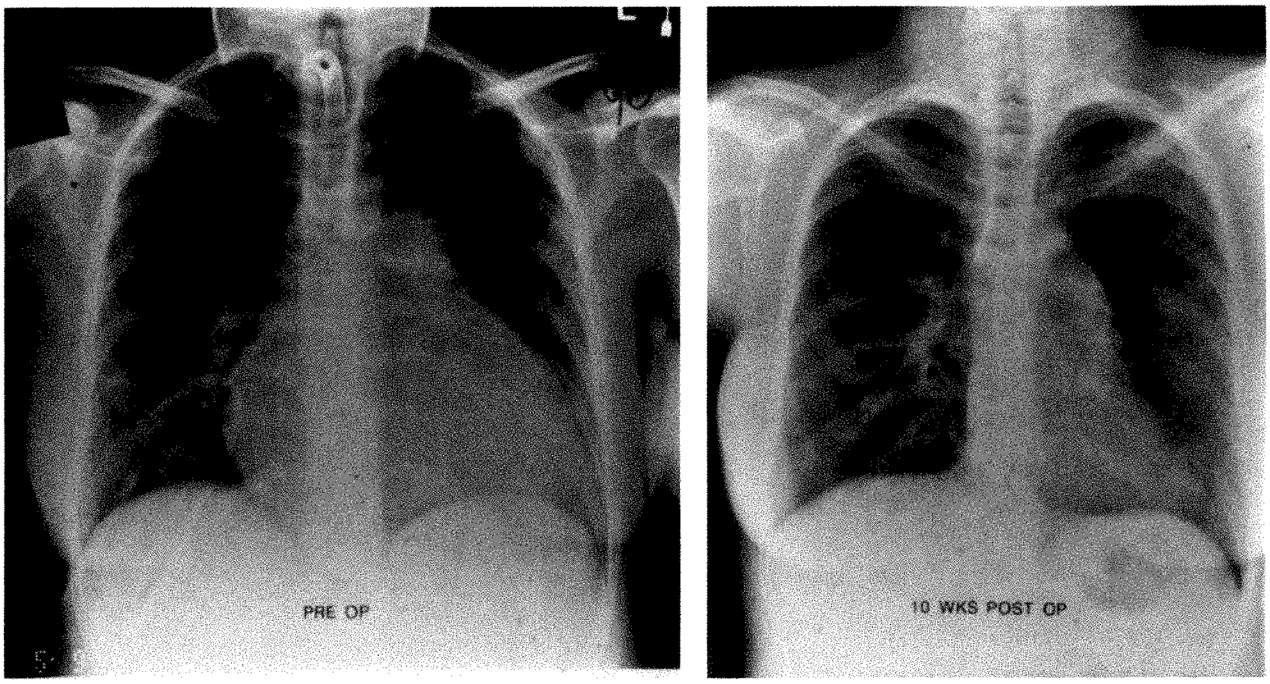

FIG. 6. Chest x-rays of a 27-year-old woman who received a right single lung transplant for primary pulmonary hypertension. Left, preoperative; right, 10 weeks postoperative.

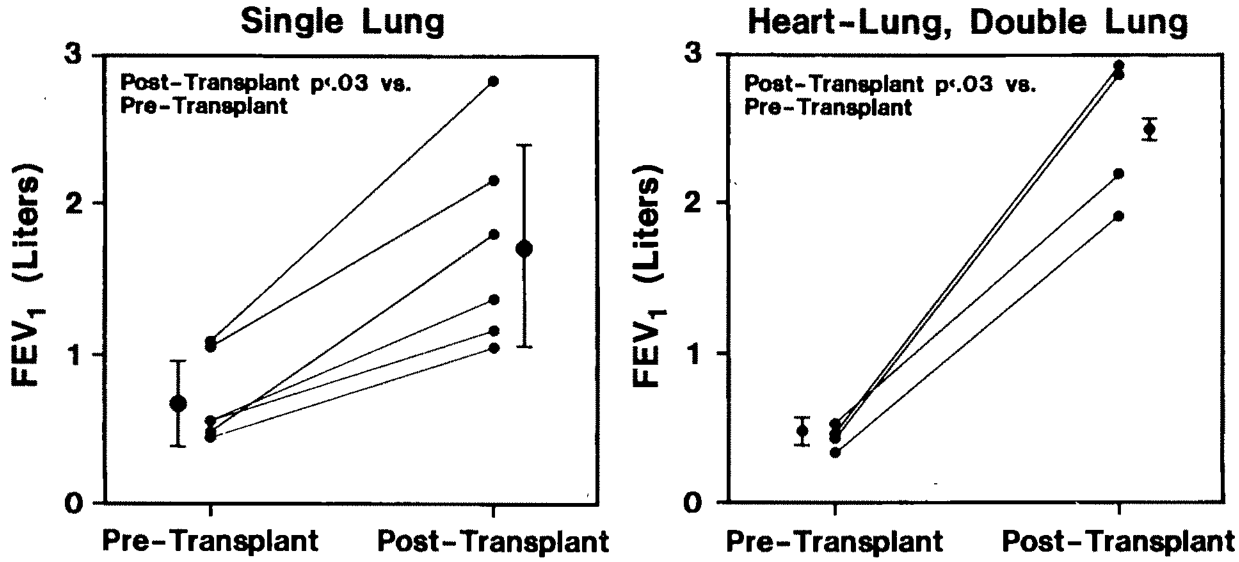

FIG. 7. Preoperative and postoperative FEV_1 in patients receiving (left) single lung and (right) heart–lung transplants for obstructive airway disease.

decrease in mPAP in patients with severe degrees of pulmonary hypertension. One instructive case is illustrated in Figure 6. This 27-year-old woman had pulmonary hypertension due to histiocytosis X. She had severe right ventricular failure and required tracheostomy for respiratory support before her transplant. She was awaiting a HLT; however it was decided to perform a SLT because of the greater likelihood of finding a donor. Single-lung transplantation was performed on cardiopulmonary bypass and she had an excellent result. Figure 6 compares her preoperative chest radiograph with one taken 10 weeks postoperatively. The heart size has returned to normal, the tracheostomy tube has been removed, and the allografted right lung appears normal radiographically.

Pulmonary Function

Three HLT and nine LT recipients (7 SLT, 1 double, 1 bilateral SLT) have undergone transplantation for obstructive airways disease (OAD). One SLT recipient died early after transplant of a pulmonary embolus. All remaining patients are long-term survivors. Examining this group in its entirety, these procedures are efficacious in improving pulmonary function as measured by forced expiratory volume in 1 second (FEV_1). Mean preoperative FEV_1 was 0.60 ± 0.25 L and improved to 2.03 ± 0.70 L after transplant ($p < 0.0001$). Figure 7 demonstrates the comparative benefit of HLT and SLT in effecting a satisfactory improvement in pulmonary function in patients with OAD. Single-lung transplant patients had a mean pretransplant FEV_1 of 0.69 ± 0.29 L. This improved to a mean of 1.73 ± 0.67 L post-transplant ($p < 0.03$), an improvement of 150%. Patients undergoing bilateral lung replacement (HLT, double, or bilateral single) had a mean pretransplant FEV_1 of 0.46 ± 0.08 L, improving to a mean of 2.49 ± 0.51 L post-transplant, an increase of over 400%.

Although patients receiving bilateral lung replacement enjoyed a significantly greater incremental increase in FEV_1 than patients receiving unilateral lung replacement (2.03 ± 0.48 L for bilateral *vs.* 1.04 ± 0.46 L for unilateral, $p < 0.03$), SLT recipients have experienced very satisfactory rehabilitation after transplantation. Figure 8 demonstrates the results of exercise testing performed in a group of five SLT and six HLT recipients. Comparing oxygen comsumption ($\dot{V}O_2$, ml/min) before and after exercise, recipients of one lung and recipients of two lungs demonstrate virtually equivalent increases in cardiovascular function.

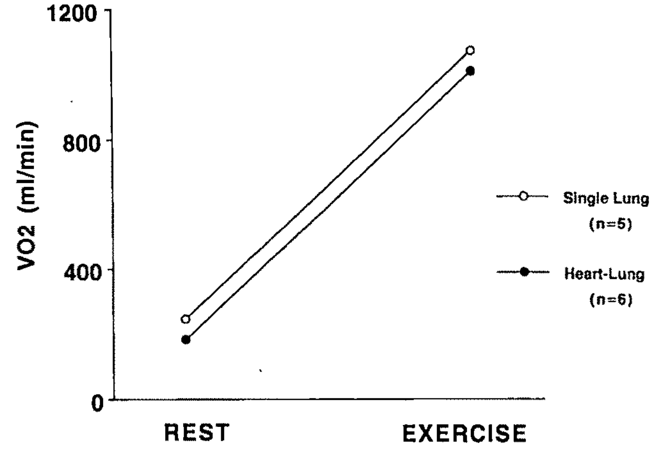

FIG. 8. Comparison of exercise testing results after single lung or heart–lung transplant (oxygen consumption, VO_2, ml/min).

Survival

Figure 9 depicts Kaplan-Meier survival of all HLT and LT patients. Survival is 82% and 73% at 1 and 2 years, respectively. Figure 10 compares survival of HLT patients with that of LT patients. Heart-lung transplant recipients experienced survival of 85% and 74% at 1 and 2 years, respectively. Recipients of LT have enjoyed survival of 80% at 1 and 2 years (p = NS relative to HLT).

Management of the Airway Anastomosis (es)

Heart-Lung Transplants. The tracheal anastomoses in the HLT recipients was not reinforced in 17 of the 23 patients. In the remaining six patients, the anastomosis was wrapped; with omentum in one and with donor pericardium in the remainder. There has been only one airway problem, an incidence of 4%. This was a contained dehiscence 4 to 5 weeks post-transplant in a 3-year-old patient. This child had been ventilator dependent for his entire life as a consequence of bronchopulmonary dysplasia. He had been receiving steroid therapy for reactive airways disease until approximately 4 weeks before his transplant. In addition he had severe tracheobronchial malacia involving the distal trachea and mainstem bronchi. The anastomosis was performed with interrupted absorbable suture material, and was wrapped with donor pericardium. He has required multiple bronchoscopies with balloon dilatation and laser therapy, and is awaiting implantation of a Silastic Y stent. Nonetheless, he is off the ventilator and walking, both for the first time in his life. His allografted organs are functioning normally.

Lung Transplants. Of 22 anastomoses in 20 LT recipients (two patients had bilateral SLT), 17 were reinforced with an omental pedicle graft traversing the diaphragm. One patient with previous abdominal surgery had the anastomosis wrapped with an internal mammary artery pedicle graft. Four anastomoses were unprotected. Anastomoses were performed in interrupted fashion with either absorbable or nonabsorbable suture material. In recent patients, an attempt has been made to telescope the donor bronchus into the recipient bronchus for the space of at least one cartilaginous ring. Of 17 anastomoses in 16 patients who have survived surgery and are at least 3 months post-transplant, there have been two that have demonstrated cicatricial narrowing. This represents an incidence of 11.7%. One was a patient, the first successful SLT in our series, who had a right SLT with bronchial omentopexy for lymphangioleiomyomatosis. She developed anastomotic narrowing at approximately 3 months after her transplant. She has had improvement and stabilization of her airway after aggressive rigid and fiberoptic bronchoscopy with dilatation and laser therapy. Somewhat disappointing has been the fact that her pulmonary function has not improved as much as expected given the restored patency of her airway. The other airway problem is in a 36-year-old man who underwent bilateral SLT for cystic fibrosis. At approximately 3 months after surgery, he developed stenosis at the site of his left bronchial anastomosis. Of interest is the fact that this very thin individual had insufficient omentum to reach both bronchial anastomoses. Therefore only the right bronchial anastomosis was wrapped with omentum, and this anastomosis is widely patent, whereas the contralateral, unprotected anastomosis has strictured. This patient has responded favorably to repeated balloon dilatation.

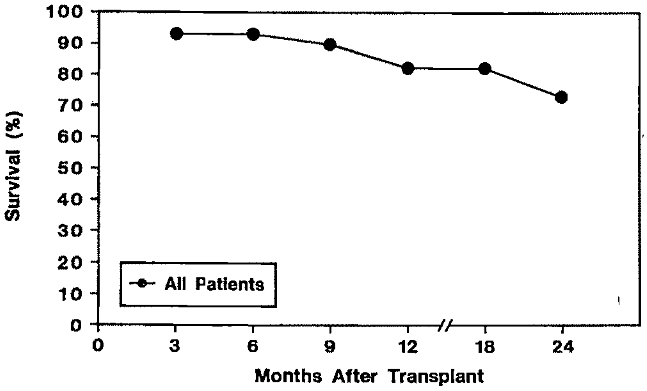

FIG. 9. Kaplan-Meier survival of all patients undergoing lung or heart-lung transplant.

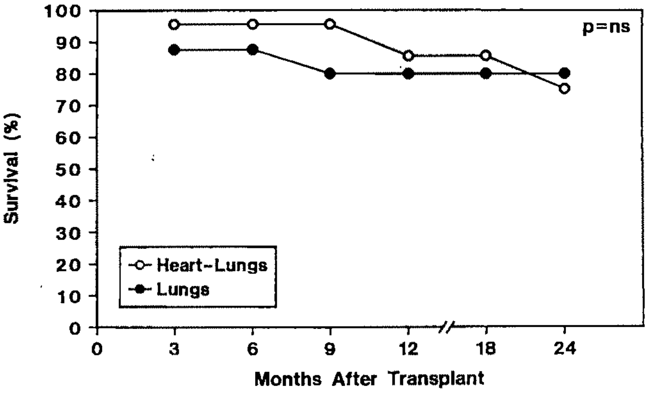

FIG. 10. Comparison of Kaplan-Meier survival of patients after lung-only and heart-lung transplant.

Other Complications

Return to the operating room for hemorrhage was required in 4 of 44 procedures (9%). One patient developed mediastinitis with a mycoplasma organism after HLT. This individual required multiple procedures for drainage of his mediastinum and pleura, but survived after a prolonged course. Another patient developed empyema after HLT. This responded to tube thoracostomy followed by rib resection with complete resolution. Two patients had herniation of abdominal viscera into the chest through the opening created for passage of the omental pedicle graft. Both patients required emergency surgery, and in one, a colostomy was necessary to decompress a massively dilated colon. Two patients sustained phrenic nerve injuries. One appeared early and plication was necessary to allow separation from the ventilator. The other case appeared several weeks after transplant. Plication was performed and resulted in an improvement in function in the allografted lung.

One patient, an HLT recipient, developed severe pulmonary edema with reperfusion of her graft, and required extracorporeal membrane oxygenator (ECMO) support for 48 hours. She was successfully weaned from ECMO, and is a long-term survivor.

Perioperative Pulmonary Dysfunction

In an attempt to improve the diagnostic accuracy of perioperative pulmonary dysfunction in patients after lung and heart–lung transplantation, a program of frequent FOB with BAL and TBB was instituted. Sixteen transplants in 15 patients were studied during a total of 28 episodes of perioperative pulmonary dysfunction. Lung allograft preservation injury occurred in five instances. There were 14 episodes of clinical lung rejection. This was confirmed by TBB histology in nine of the cases. Infectious causes were elicited in 12 instances. Of note is the fact that bronchoscopy yielded information not expected from the initial evaluation in 10 instances (39% of the episodes). Employing the above studies for indications including new onset of pulmonary symptoms, increase in the alveolar-to-arterial oxygen gradient, or any change in the chest radiograph, this approach yielded a sensitivity of 79% and a specificity of 100% in the diagnosis of perioperative pulmonary dysfunction.[10]

Obliterative Bronchiolitis

Five patients have developed obliterative bronchiolitis (OB) after HLT (23% of survivors), and all have died at intervals of 1 to 2 years post-transplant. Three patients after SLT (19% of survivors) have evidence of OB within 3 to 10 months of transplant, and one patient has died. Of particular interest is the fact that all but one of these eight patients had pulmonary hypertension as the underlying diagnosis necessitating their transplant. Four of the five HLT and all three SLT recipients had primary or secondary pulmonary hypertension before transplantation. The single exception occurred in a 53-year-old lady with emphysema due to A1ATD. This lady underwent HLT and expired from OB at 1 year post-transplant.

Discussion

The concept of transplantation of pulmonary tissue as a therapeutic modality in the treatment of end-stage lung disorders was introduced by the successful engraftment of combined heart–lung blocks, first reported by Reitz et al.[1] As catalogued so well by Cooper et al.,[2] previous attempts at transplanting the lung had resulted in a litany of failures, due almost exclusively to inadequate healing of the airway anastomosis.[11] The cases reported by Reitz et al. represent a landmark in the annals of thoracic transplantation. Airway healing was uniform, and long-term survival with allografted lungs was shown to be feasible. Although plagued with problems of insufficient donors and the development of recurrent respiratory insufficiency due to bronchiolitis obliterans, heart–lung transplantation indisputably provided the latticework on which all subsequent developments in pulmonary transplantation have become interwoven.

The indications for lung and heart–lung transplantation continue to evolve. Certain trends, however, are beginning to emerge. The data in the current series regarding waiting times before transplant indicate a strong advantage in favor of single-lung *versus* heart–lung transplantation. Patients awaiting SLT waited 150 days, compared with 384 days for those awaiting HLT. This clear trend will have increasing ramifications for patient selection in the future. Individuals awaiting bilateral lung replacement can expect a longer wait before undergoing transplantation; however the wait still should be substantially shorter than that accompanying HLT. The explosion in the number of hospitals performing heart transplantation has virtually precluded the possibility of obtaining heart–lung blocs for transplantation, because the demand for suitable donor hearts has increased so dramatically that most donor hearts are implanted in waiting heart recipients. Lung transplantation, either single or bilateral, has demonstrated its ability to benefit patients with an increasingly wide range of pulmonary disorders. Originally introduced to treat patients with primarily restrictive pulmonary diseases,[2,11,12] lung transplantation has now been applied successfully to patients with obstructive diseases,[13,14] pulmonary sepsis,[7,15] and pulmonary vascular disease,[16,17] to name a few. These latter pathologies formerly were thought to require combined heart and lung replacement instead of lung-only replacement.

The Operations

The program at the University of Minnesota has had experience with 23 heart–lung and 21 lung transplant procedures. Certain technical features have become apparent that improve the safety of these operations. In the HLT procedure, we have found it expeditious to employ the TA-90 stapler to divide the pulmonary hila. This not only facilitates recipient pneumonectomy, but improves hemostasis as well. There has been only one death within 30 days in our 23 heart–lung recipients, an operative mortality rate of 4%. All other patients have been long-term survivors.

In the lung transplant recipient, it is important to have an intimate familiarity with the various methods of instituting cardiopulmonary bypass. Although many procedures can be performed without the need of extracorporeal support of the heart and lungs, in those cases where this support is necessary, the surgeon must be able to expeditiously institute cardiopulmonary bypass in a safe and effective manner. Situations that mandate bypass include hypercarbia with acidosis that cannot be controlled with buffers, hypoxia below 50 mm Hg, and any sign of circulatory instability. One maneuver that we have found helpful is to encircle the ipsilateral pulmonary artery as soon as possible after the lung is deflated after clamping of the endotracheal tube to that lung. This prevents shunting of a large amount of desaturated blood to an unventilated lung, and the attendant hypoxemia. In the child or the small adult, cardiopulmonary bypass requires cannulation of structures in the chest, and planning of the operation must take this into consideration. The choice of incision for lung transplantation is decided based on several factors. Single-lung transplantation in the adult is best performed through an anterolateral thoracotomy, with the ipsilateral femoral vessels available, should bypass be necessary, as described by Cooper et al.[2] In the child, the best approach is determined by body size, the likelihood of needing extracorporeal circulation, previous surgery, and the presence or absence of a tracheostomy. It behooves the transplant surgeon to be comfortable with a variety of approaches, because some ingenuity is required to choose the best approach for a given situation. The bilateral transverse thoracotomy described by Pasque et al.[7] is of great utility in a variety of circumstances. This incision provides excellent exposure to the structures of the mediastinum, as well as to the lungs, pleural spaces, and pulmonary hila. This approach has been employed in our series for bilateral SLT, as well as for right and left SLT. In those instances in which bilateral transverse thoracotomy has been used for SLTs, there has been the need for concomitant cardiopulmonary bypass, in one case for pulmonary hypertension and in another for associated repair of an intracardiac defect associated with pulmonary hypertension. The other option that should not be overlooked for SLT is that of midline sternotomy. This incision provides good exposure to the right pulmonary hilum for right SLT, and institution of cardiopulmonary bypass (CPB) is straightforward, should it be needed. Using the operative techniques described, we have had four perioperative deaths in the first 21 lung transplant procedures.

Pediatric Lung and Heart–Lung Transplantation

The pediatric lung and heart–lung transplant series reported herein represents the largest experience reported to date. Several lessons have already been learned in this group of patients. Most importantly, both lung and heart–lung transplantation are technically feasible in children at least as young as 3 years (HLT) and 4 years (SLT), respectively. Cardiopulmonary bypass appears to be more uniformly required to achieve safe transplantation in the pediatric age group. Furthermore CPB can less reliably be effectively instituted through the femoral route in children. Therefore the planning of the operative approach must allow for the possibility of CPB through the chest. Retransplantation, either early or late after the initial procedure, has proven technically feasible, if somewhat difficult. Five of our 7 patients are alive 4 to 22 months after transplant. One is still receiving treatment for cicatricial narrowing at the tracheal anastomosis (HLT), but is doing very well overall with excellent graft function. This patient had been ventilator dependent and on steroid therapy for the entire 3 years of his life before transplant, and is currently off the ventilator and walking, both for the first time in his life. Another, a 4 year-old SLT for PPH, has developed OB, and is awaiting retransplant. Another child, an HLT at age 5 for lymphocytic interstitial pneumonitis, has had a lymphoproliferative process in one of his allografted lungs that has responded well to alpha-interferon and gamma-globulin. He has a recurrent infiltrative process in his lungs, which on biopsy appears very similar to his presenting pathologic process. The remaining two patients are well, without evidence of OB or recurrent disease.

Pulmonary Artery Pressures

The data presented herein indicate that lung and heart–lung transplantation are equally effective in the treatment of elevated pulmonary artery pressures due to primary pulmonary hypertension. Limited experience also suggests that the two procedures are efficacious in reversing elevated pulmonary artery pressures occurring secondary to a congenital heart defect with increased pulmonary blood flow (Eisenmenger's syndrome). The experience gained in this group of patients has provided critical information regarding the ability of SLT to replace HLT in the treatment of individuals with pulmonary hypertension from a variety of causes. The knowledge that SLT and HLT are equally effective in reversing elevated pulmonary artery pressures provides a major incentive to apply SLT in these cases, because many more patients can potentially benefit from SLT, for any given number of donors. An area of great interest remains the application of repair of congenital heart defects in conjunction with SLT. This operation has been successfully accomplished for simple defects (patent ductus arteriosus).[17] For more complex defects, however, the utility of this approach remains speculative. The case we report of a VSD repair and concomitant SLT proved quite feasible technically, and was accomplished with a very acceptable period of allograft ischemia. Nonetheless this individual succumbed to a combination of low cardiac output and a poorly functioning donor lung. Clearly the right ventricular pathology proved more complex than we had appreciated, and the postoperative course was greatly complicated by the development of right-ventricular outflow tract obstruction and low forward cardiac output. No doubt this group of patients can benefit from SLT; however selection must be carefully performed, and cardiac evaluation must be scrupulously thorough, so that all cardiac defects may be corrected at the time of transplant.

Pulmonary Function

In patients afflicted with pulmonary parenchymal diseases, the goal of lung replacement is to increase pulmonary function and to improve exercise capacity. Cooper, Patterson, and others have reported the dramatic reversal in lung dysfunction that can accompany successful lung transplantation, initially in patients with pulmonary fibrosis.[2,11,12] Until recently there was concern that OAD would require bilateral lung replacement. This concern related to fears of overinflation of the remaining native lung with compression of the transplanted lung, if single-lung replacement were performed. Several authors have now reported successful SLT for OAD, and the worries regarding problems with the native lung have proven largely unfounded.[14,18] In the current series, there are 12 patients who received transplants for OAD. Three patients received HLT and nine, LT, either single or bilateral. Both LT and HLT proved efficacious in providing increased lung function after transplantation. As expected, patients receiving two lungs experienced greater improvement in lung function than those receiving one lung (400% *vs.* 150% increase in FEV_1). Nonetheless SLT recipients have enjoyed cardiovascular rehabilitation after transplantation that is virtually indistinguishable from that experienced by recipients of bilateral lung replacement.

Survival

A critical factor in any comparison of procedures is that of survival. Clearly if one procedure proves vastly superior in this vital outcome area, this single fact would outweigh many lesser advantages of any procedure with which it is being compared. Survival after any lung transplant procedure in the current series is 82% and 73% at 1 and 2 years, respectively. Patients undergoing HLT have Kaplan-Meier survival of 85% and 74% at 1 and 2 years, respectively, compared with 80% at 1 and 2 years for recipients of LT ($p =$ NS). Data in both groups are intermediate, at best, because the majority of procedures have been performed in the past 2 years. Nonetheless lung and heart–lung transplantation appear to be very similar relative to patient survival in the short-to-intermediate term after transplantation.

Airway Anastomosis

The airway anastomosis was, for many years, the primary obstacle to successful engraftment of transplanted lungs. Work by the Toronto group resulted in the use of the omental pedicle graft to reinforce the bronchial anastomosis, and to allow ingrowth of new blood vessels to this poorly vascularized structure.[19,20] This work, coupled with the demonstration by Reitz et al. that long-term survival with allografted lungs was feasible,[1] brought closer the day of successful single lung transplantation. The availability of improved immunosuppression in the form of cyclosporine was also highly instrumental in allowing successful engraftment of both lung and heart–lung grafts. Thus a combination of factors converged to eventuate in the realization of the elusive dream of transplanting the human lung. It remains our preference to employ the omentum whenever it is available for wrapping the airway anastomosis in lung or bilateral lung transplants. In heart–lung transplant procedures, the anastomosis is wrapped with donor pericardium when it is available. Most HLT anastomoses have not been reinforced. Attempts also are made to telescope the donor bronchus into the recipient bronchus for the distance of one cartilaginous ring, after the method described by Veith et al.[21] and popularized by Trinkle.[16] This is particularly important when the omentum is not available.

Other Complications

As expected, surgery of the magnitude of lung or heart–lung transplantation is attended by complications. Nine per cent of patients required return to the operating room for hemorrhage. All these patients had required cardiopulmonary bypass for the performance of their transplant (2 SLT, 1 HLT, 1 bilateral SLT). Two patients have developed intrathoracic infections, one early and one late, both after HLT. Both cases responded to aggressive drainage and antibiotic therapy. Gratifyingly there have been no infectious deaths in the entire series. The omental pedicle graft has proven to be a mixed blessing in our hands. Two patients have had herniation of transverse colon into the left chest through the opening made to pass the omentum. Both have required reoperation to repair the diaphragmatic defect, and one required colostomy to decompress a massively dilated transverse colon and cecum. Phrenic nerve injury also has occurred and has been associated with significant morbidity and prolongation of the hospital course.

Perioperative pulmonary dysfunction can present either early or late after transplant. Two HLT recipients have required ECMO in the early postoperative period for pulmonary dysfunction. One was successfully weaned from ECMO support at 48 hours, and is a long-term survivor. The other individual had massive, uncontrollable hemorrhage necessitating large-volume transfusion of blood products. This resulted in pulmonary edema, and ECMO had to be employed. This patient died at day 4 of ongoing, uncontrolled hemorrhage, despite multiple reoperations, arteriography, and attempted embolization of posterior mediastinal bleeding points. Pulmonary dysfunction occurring later has responded to an aggressive diagnostic and therapeutic protocol based on frequent FOB, combined with TBB and BAL. Liberal use of these modalities has yielded a sensitivity of 79% and a specificity of 100% in the diagnosis of perioperative pulmonary dysfunction.[10]

Obliterative Bronchiolitis

Obliterative bronchiolitis (OB) has long been recognized as the main impediment to long-term survival after heart–lung transplantation. Despite premature claims to the contrary, however, it is now clear that recipients of lung grafts can expect to experience an approximately equal incidence of this devastating disorder. LoCicero et al.[22] describe an unequivocal case of OB occurring 9 months after SLT in a patient who eventually died of this disorder.[22] McGregor et al.[23] also have reported the occurrence of OB after isolated SLT. In the current series of patients, OB has occurred in five patients after HLT (23%), and in three long-term survivors of LT (19%). All patients developing OB after HLT have died as a result of this process. One of the three LT patients with OB has died, and the remaining two are awaiting retransplant. Of particular interest is the fact that 7 of the 8 patients who have developed OB have had pulmonary hypertension as the diagnosis necessitating transplantation.

Summary and Conclusions

Lung and heart–lung transplantation are established therapeutic modalities for the treatment of end-stage pulmonary and cardiopulmonary disorders. The current experience at a single institution discloses certain information of importance to the field of thoracic transplantation. Lung transplantation, either single or bilateral single, can safely and effectively be applied to diseases formerly thought treatable only by heart and lung replacement. Most notable among these diseases are the obstructive airways diseases and diseases involving irreversible elevation of the pulmonary artery pressures. The operative safety of lung transplantation compares favorably with that of heart–lung transplantation. Furthermore the incidence of airway complications, although somewhat higher after LT, is not prohibitively so. Indeed, Levine, of Trinkle's group has reported successful SLT in a significant number of patients without the use of an omental pedicle graft with no airway complications.[16] A point that has emerged very clearly is the fact that patients awaiting lung transplants can expect a far shorter waiting time before transplant than those awaiting heart–lung transplants. Also because three individual recipients can potentially benefit from a single donor with donation of each of the two lungs as well as the heart, lung transplantation results in a better usage of scarce donor organs. For all these reasons, lung transplantation should be the procedure of choice whenever possible for individuals with end-stage lung disease. Heart–lung replacement still has a role in the treatment of patients with combined heart and lung disease in situations in which the heart is unable to be repaired.

References

1. Reitz BA, Wallwork JL, Hunt SA, et al. Heart-lung transplantation: successful therapy for patients with pulmonary vascular disease. N Engl J Med 1982; 306:557–564.
2. Cooper JD, Pearson FG, Patterson GA, et al. Technique of successful lung transplantation in humans. J Thorac Cardiovasc Surg 1987; 93:173–181.
3. Metras H. Note preliminaire surj la greffe totale du poumon chz le chien. CR Acad Sci (Paris) 1950; 231:1176–1178.
4. Hardin CA, Kittle CF. Experiences with transplantation of the lung. Science 1954; 119:97–98.
5. Patterson GA, Cooper JD, Dark JH, et al. Experimental and clinical double lung transplantation. J Thorac Cardiovasc Surg 1988; 95: 70–74.
6. Patterson GA, Todd TR, Cooper JD, et al. Airway complications after double lung transplantation. J Thorac Cardiovasc Surg 1990; 99:14–21.
7. Pasque MK, Cooper JD, Kaiser LR, et al. An improved technique for bilateral lung transplantation: rationale and initial clinical experience. Ann Thorac Surg 1990; 49:785–791.
8. Bolman RM, Olivari MT, Sibley R, et al. Current results with triple therapy for heart transplantation. Transplant Proc 1987; 19:2490–2491.
9. Baumgartner WA, Traill TA, Cameron DE, et al. Unique aspects of heart and lung transplantation exhibited in the 'domino-donor' operation. JAMA 1989; 261:3121–3125.
10. Burdine J, Hertz MI, Snover DC, Bolman RM. Heart-lung and lung transplantation: peri-operative pulmonary function. Transplant Proc 1991; 23(1):1176–1177.
11. Grossman RF, Frost A, Zamel N, et al. Results of single-lung transplantation for bilateral pulmonary fibrosis. N Engl J Med 1990; 322:727–733.
12. Toronto Lung Transplant Group. Unilateral lung transplantation for pulmonary fibrosis. N Engl J Med 1986; 314:1140–1145.
13. Yacoub M, Khaghani A, Theodoropoulos S, et al. Single-lung transplantation for obstructive airway disease. Transplantation Proceedings. 1991; 23(1):1213–1214.
14. Mal H, Adreassin B, Pamela F, et al. Unilateral lung transplantation in end-stage pulmonary emphysema. Am Rev Respir Dis 1989; 140:797–802.
15. Cooper JD. The evolution of techniques and indications for lung transplantation. Ann Surg, 1990; 212:249–256.
16. Levine SM, Gibbons WJ, Bryan CL, et al. Single lung transplantation for primary pulmonary hypertension. Chest 1990; 98:1107–1115.
17. Fremes SE, Patterson GA, Williams WG, et al. Single lung transplantation and closure of patent ductus arteriosus for Eisenmenger's syndrome. J Thorac Cardiovasc Surg 1990; 100:1–5.
18. Patterson GA. Lung transplantation for chronic obstructive pulmonary disease. Clin Chest Med 1990; 11(3):547–554.
19. Dubois P, Choiniere L, Cooper JD. Bronchial omentopexy in canine lung allotransplantation. Ann Thorac Surg 1984; 38:211–214.
20. Lima O, Goldberg M, Peters WJ, et al. Bronchial omentopexy in canine lung transplantation. J Thorac Cardiovasc Surg 1982; 83: 418–421.
21. Veith FJ, Kamholz SL, Mollenkopf FP, Montefusco CM. Lung transplantation 1983. Transplantation 1983; 35:271–278.
22. LoCicero J, Robinson P, Fisher M. Chronic rejection in single-lung transplantation manifested by obliterative bronchiolitis. J Thorac Cardiovasc Surg 1990; 99:1059–1062.
23. McGregor CGA, Dark JH, Hilton CJ, et al. Early results of single lung transplantation in patients with end-stage pulmonary fibrosis. J Thorac Cardiovasc Surg 1989; 98:350–354.

DISCUSSION

DR. B. GRIFFITH (Pittsburgh, Pennsylvania): Thank you, Dr. Thompson, members, and guests. It is a privilege to be invited to comment on the paper by Dr. Bolman and his colleagues. We looked with a certain degree of envy on the accomplishments of this group.

I have a number of questions to pose to Dr. Bolman. The questions relate to the relative indications currently in his mind for the various procedures, the use of omentum with respect to all cases of either bilateral or single-lung transplantation, his incidence of obliterative bronchiolitis, and relative issues of immune suppression that he was not able to comment on due to time limitations.

With respect to heart–lung transplantation, it appears that the indication currently rests on the degree of left or right heart dysfunction, the presence or absence of coronary artery disease, and the relative complexity of congenital heart disease.

As he points out today, the new indication might be the relative medical urgency as we all now believe, and Dr. Bolman has shown clearly that the relative availability of a lung donor or double lung donor appears to be far greater than that of a combined heart and lung donor.

With respect to single- and double-lung transplantation, the only absolute indication for double-lung transplantation might be septic lung disease, such as cystic fibrosis.

I am concerned that the objective evidence might be less than compelling from my viewpoint with respect to obstructive lung disease and single-lung transplantation.

Pulmonary function tests shown today demonstrate limited reserve with respect to single-lung transplantation for obstructive lung disease, and I am concerned that the O_2 consumption demonstrated might more belie poor conditioning as opposed to absolute functional parity between double- and single-lung transplantation.

We believe that the omentum has been overused. You have shown in your paper that there have been two complications. I wonder whether your current viewpoint is whether it continues to be an absolute indication. We do not use it routinely and do not appear to have more difficulties.

Obliterative bronchiolitis is a problem, and it relates to immune suppression and/or other diseases, as you have shown with respect to your heart program, viral infection, and coronary artery disease. There may be a parallel. We believe that viral disease might up-regulate the allogenic response and cause coronary artery disease; it also may be related to obliterative bronchiolitis.

Your immune suppressive protocol is the classically developed triple-drug therapy, and although you mentioned in the article some use of antilymphocyte globulin in a prophylactic sense, I wish that you would comment further on whether you see any differences.

Very few of your patients have died of infectious disease, and in transplantation we know that when no one has died of infection, perhaps we have not provided sufficient immune suppression.

Thank you very much, Dr. Bolman. I appreciate your letting me review your data.

DR. KEITH REEMTSMA (New York, NY): This is a very timely report that Dr. Bolman has shared with us, and I think the key word in the title is "evolution." He has appropriately emphasized that this is a time of changing indications and changing methods in the replacement of lungs.

It is worth recalling that the first clinical experience in lung transplantation was reported by a former president of this Association, Dr. James Hardy, and that the two major milestones that Dr. Bolman referred to, the introduction of heart–lung transplants by Bruce Reitz, and the demonstration of the feasibility of single-lung transplants by Dr. Joel Cooper, were both made by members of this Association. It is particularly fitting that Dr. Bolman present this work the day after his election to membership in this Association.

The authors have made a convincing case that single-lung transplantation now may be appropriately applied to conditions that, until two years ago, were reserved for heart–lung transplantation. These are specifically primary pulmonary hypertension and Eisenmenger's complex. Dr. Bolman has certainly demonstrated that the logistics of single-lung transplant makes the waiting time shorter. He is able to provide, through the use of multiple organs, an expanded pool of organs for patients who may be helped by these procedures.

One of the interesting observations in the manuscript that Dr. Bolman did not have a chance to comment on in his presentation is the fact that seven of eight of their patients with the very troublesome complication of obliterative bronchiolitis were patients in whom the original condition was pulmonary hypertension. I would ask Dr. Bolman, in his closing comments, if he might reflect on whether he thinks there is a causal relationship between these.

Finally I believe it is appropriate that we give recognition to Dr. Najarian and his colleagues, such as Dr. Bolman, at the University of Minnesota who, over the past several decades, have provided us exemplary leadership in the field of transplantation, an area that will be extremely important in the next decade and into the next century. Thank you.

DR. F. GROVER (San Antonio, Texas): Since March of 1988, our lung transplant group in San Antonio, headed by Dr. Kent Trinkle, with the participation of Dr. John Calhoun and myself, has performed 44 single-lung transplants in 41 patients, with 11 deaths, eight of which were CMV related and five of which were early. The 1-year actuarial survival is 78%; in the 2-year, 73%.

Although we started out, as most groups performing the majority of these procedures for pulmonary fibrosis, we sought to expand the indications for single-lung transplantation to avoid heart–lung transplantation when possible, as you also have done.

In January of 1989, we performed our first single-lung transplant for chronic obstructive pulmonary disease and have now performed 24 for this indication, of which 13 were alpha-1 antitrypsin deficiencies. Seven patients were operated on for a variety of other conditions, including idiopathic pulmonary fibrosis, 3 for PPH, 1 for Eisenmenger's, 2 for sarcoid, 2 for scleroderma, and 2 for pulmonary fibrosis secondary to chemotherapy. Our waiting time for single-lung donors is also short, averaging from 3 to 4 months.

Of concern to us are our results in the pulmonary hypertension group. Ten of our patients had pulmonary hypertension defined as a PA pressure or pulmonary vascular resistance of two-thirds systemic. Five were reactive to nitroprusside, and five were fixed. The five patients who had reactive pulmonary hypertension did well, but four of the five with fixed pulmonary hypertension died, including two of the three with PPH and one with Eisenmenger's. Of those who survived the initial 30 days, it appeared that they did well for the initial 6 months, but then in the 6- to 9-month period they began to deteriorate with a combination of infection and rejection with findings of bronchiolitis obliterans and pulmonary fibrosis. We question whether this may be related to the increased pulmonary blood flow to the transplanted lung.

I am curious if your group has experienced difficulty with the subgroup of patients, as has ours.

Eight of our eleven deaths have been CMV related. Because of this we have eliminated cytolytic therapy and now prophylactically treat all of the patients with Ganciclovir® and Sandoglobulin® during the perioperative period. Since then we have had a marked decrease in the incidence of CMV infection and what appears to be a decrease in the incidence of rejection, inferring that the immunologic stimulus of the CMV may be a factor in the associated rejection.

What is your immunosuppressive regimen? What is your incidence of CMV? And have you noted an association between CMV infection and rejection?

I would like to add that all of our single-lung transplants have been done with no significant bronchial complications, in spite of two thirds of the patients being on preoperative prednisone, the use of perioperative steroids, and no wrapping techniques.

We certainly agree with your conclusions that single-lung transplantation offers better use of donor organs with as good or better results than heart–lung transplantation and with much shorter waiting times in selected cases. It offers excellent palliation for those patients with end-stage pulmonary parenchymal disease, but we believe that more experience is needed before final judgment can be made regarding its efficacy for patients with irreversible pulmonary hypertension.

DR. H. VILLAR (Tucson, Arizona): We have two patients in whom we have had complications developed. In both of them, cystic fibrosis was the indication for lung transplant. And I wonder, Dr. Bolman, if you think that perhaps because of the systemic involvement of cystic fibrosis, this may be perhaps a contraindication to lung transplants.

DR. R. MORTON BOLMAN (Closing discussion): Dr. Griffith, it is an honor to have our work discussed by someone who has made as many contributions to the field of thoracic transplantation as you have.

Regarding indications I believe this is still an area of evolution. Because of donor limitations we currently are employing single-lung transplantation for the majority of our patients with obstructive airway disease. Some would argue that a young individual with obstructive airway disease would be better off receiving bilateral lung transplants. As we demonstrated although there is greater improvement in pulmonary function testing in individuals receiving two lungs as opposed to one, this does not necessarily translate into a significant benefit in exercise capacity.

With respect to the need for omental wrapping, we have chosen to employ the omentum whenever it is available. We have, however, had two diaphragmatic hernias requiring operation for repair. Therefore the use of the omentum is clearly not without its problems. Also Dr. Trinkle and his colleagues have shown very nicely that omental wrapping may not be necessary even in patients receiving preoperative steroid therapy. Nonetheless we continue to recommend its use when available.

Immunosuppression in our patients is based on triple therapy consisting of cyclosporine, Imuran®, and prednisone. We have used Minnesota antilymphocyte globulin in most of our patients for 3 to 5 days immediately after transplantation. We have observed no difference in the incidence of CMV, and no difference in the incidence of rejection between those patients receiving and not receiving antilymphocyte globulin (ALG). We have had no infectious deaths in our experience to date in recipients of lung and heart-lung allografts. This has caused us to speculate whether we are administering adequate immunosuppression and whether we might be running the risk of long-term bronchiolitis obliterans. Although no one has yet demonstrated a relationship between immunosuppressive regimen and bronchiolitis obliterans in pulmonary transplantation, this remains a concern. We have some very interesting data showing that obliterative bronchiolitis in fact may be related to certain growth factors elaborated by the lungs in response to injury. Platelet-derived growth factor has been identified in excess quantities in lavage fluid from patients with obliterative bronchiolitis after lung transplantation by our pulmonary medicine group.

Dr. Reemtsma, thank you for your comments. It is a privilege to have you discuss our work, because you are an individual whom I admire more than almost anyone in the field of surgery. The indications and contraindications in pulmonary transplantation are not yet fully established. The high incidence of bronchiolitis obliterans observed in patients transplanted for pulmonary hypertension is a very concerning phenomenon. In fact all three single-lung recipients transplanted for primary pulmonary hypertension have developed obliterative bronchiolitis, one as soon as 2 months after transplant. We do not at this point have any indication as to what the causative relationship may be.

Dr. Grover, you and Dr. Trinkle and your group have made many contributions to the field of lung transplantation, including the demonstration that omental wrapping is not essential to successful lung outcomes, the first successful single-lung transplants for primary pulmonary hypertension, as well as obstructive airway disease and others. Thank you for your comments. We share your concern about pulmonary hypertension as an indication for single-lung transplantation. We have several more individuals awaiting this procedure and we are taking a wait-and-see approach, but this may not be an appropriate indication.

We have had no CMV deaths in our experience. We have a high incidence of CMV syndrome, in excess of 50%, but so far have not had any deaths. For that reason we have been reluctant to employ Ganciclovir® in a prophylactic capacity because this is the only agent we have available to treat these patients, and the emergence of resistant strains would be a catastrophe.

Dr. Villar, we do not feel at present that cystic fibrosis represents a contraindication to lung transplantation. Our experience is small and we have to rely on the larger experiences of other centers. We have one heart-lung and one bilateral single-lung recipient, both of whom have done well and have not had gastrointestinal complications. Clearly transplantation in the cystic fibrosis population contradicts many of the tenets of transplantation, but the outcomes being reported by centers in this country and abroad justify the continuing exploration of lung transplantation as a therapeutic modality for these unfortunate individuals.

Evolving Experience With Mechanical Circulatory Support

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Since 1985 total mechanical circulatory support for mortally ill transplant candidates has been progressively integrated into the authors' program. During this period 379 patients underwent transplantation. Of this group of patients, 62 required some form of mechanical support other than the intra-aortic balloon pump. Because intra-aortic balloon pump assist was limited in therapeutic effect and was associated with patient immobility and line-related sepsis, the next logical step toward support was the artificial heart. Of 20 patients implanted with the Jarvik heart, 17 underwent transplantation, but only 9 of these survived to discharge. In 1988, the authors abandoned the preferential use of the total artificial heart because of excessive cumulative probability of death from wound infection. They began to use the Novacor electrical assist device with the percutaneous power cord because they believed that univentricular support would be adequate for most patients, because its heterotopic position would reduce the likelihood of infection, and because it had the potential for chronic implantation. Twenty-three patients with biventricular failure (right ventricular ejection fraction <20%, 18/23) received the electrical assist device for an average of 50.4 days (range 1–193 days). All 17 transplanted patients survived until discharge. Only one of the five deaths that occurred after implantation, but without transplantation, was due to infection (candidiasis). Remarkably, all patients who survived the perioperative period ultimately survived with univentricular support alone. Based on this experience, survival of mechanically supported patients is now comparable to that of those less mortally ill.

From the University of Pittsburgh Artificial Heart Program, Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

IN THE PAST decade, as experience increased, innovative strategies have been required for dealing with progressively more complex and desperately ill candidates for cardiac transplantation. A major challenge has been supporting patients who would die before transplantation if they received only traditional therapy. This report covers three periods of our experience as specific therapeutic problems arose and as our use of mechanical circulatory support evolved. This experience has resulted in a better understanding of proper patient selection, applicability of the device, and suggested future directions for mechanical circulatory support.

Early Experience: 1980–1985

Cardiac transplantation at the University of Pittsburgh was resumed in 1980.[1] In 1981 Thomas Starzl brought expertise and early access to cyclosporine. The multi-organ program grew rapidly and soon included a large number of mortally ill patients.[2] As advocated by Reemtsma and associates,[3] to maintain life before transplantation, we used the intra-aortic balloon pump in 26 patients, with an 8% 30-day mortality rate. This compared favorably with a 17% mortality rate in patients transplanted electively or when they were receiving only intravenous inotropic support. The lower mortality rate in apparently higher-risk patients was due to the deaths of many candidates on balloon assist. Intra-aortic balloon pump assist was limited in therapeutic effect and associated with obligatory immobility of the patient, line-related sepsis, and arterial complications. During this early experience, the average waiting period for a donor heart increased from 4 days to 4 weeks. The pool of candidates was growing, but the number of donors did not keep pace, and the balloon assist did not provide adequate long-term support. Total circulatory support seemed to be a logical next step.

Total Artificial Heart: 1986–1987

The pioneering use of the total artificial heart (TAH) by DeVries[4] and associates excited many, but hopes for

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satisfactory quality of survival faded because of near universal infection and thromboembolism. Their experience suggested that the device might be more appropriate for use as a bridge to transplantation. In 1985 Copeland first used the Jarvik TAH for this purpose and several months later we began an experience that included 20 patients over the next 2 years.[5–7] Before implantation these patients required inotropic drugs for a median of 12 days (range, 2–83 days), and 17 also required support by an intraaortic balloon for a median of 2 days (range, 2–65 days).

These patients were judged not likely to survive a few hours, and once the decision to implant the TAH was made, the procedure became an emergency and was considered by us to be heroic resuscitation. A more elective use of the TAH appeared unwarranted in view of the results of the chronic trial. Infection and stroke, known to be associated with use of this device, argued for transplantation as soon as possible and priority was given for the next available donor organ. This resulted in a short duration of support, which averaged 9 days (2–48 days) and a high percentage (89%) of patients undergoing transplantation (Table 1), some in spite of lingering grave congestive heart failure.

Of the 20 recipients of the TAH, 3 died before transplantation, 1 with fungal sepsis, 1 with hemorrhage from a torn pulmonary arterial anastomosis, and 1 from multiorgan failure. Thus 17 patients received cardiac allografts. Nine of these (53%) returned home well. Eight patients died during their initial hospitalization, a 40% 30-day mortality rate. Three died of acute allograft dysfunction (two with histologically documented hyperacute rejection). The remaining five died from complications of infectious mediastinitis. Only one instance of mediastinitis was recognized before transplantation. None of these infections appeared to be related to ascending infection from the exit site of the drive line. Infection of the wound was due to enteric organisms in five, and to fungus in the other.[6] These organisms were found also in the lungs, and we believe seeding of the wound occurred through the blood or by direct translocation. Experimental work suggests that the polyurethane and orthotopically placed TAH destroys host defenses against infection.[8]

Although the many failures were discouraging, the few successes reinforced our belief in the potential for temporary and possibly permanent mechanical circulatory support. Multidisciplinary interest and institutional backing had developed, and a team of engineers, technicians, and surgeons were trained in the use of support devices.

Our experience with the total artificial heart taught us several important lessons. First that our selection of patients for the TAH with seemingly clinically innocent or unrecognized infection and their urgent transplantation led to poor results. Second there was an ill-defined intolerance of the large foreign body in the pericardial sac. Finally use of devices to bridge to transplantation lengthened the queue for organs and was flawed unless the device had potential for long-term implantation and support.

Novacor Device: 1988 to Present

An alternative approach to the use of the TAH for mechanical circulatory replacement is that of using heterotopic ventricular assist devices (VAD), either paired for biventricular or individually for univentricular support. In September 1984 Portner et al.[9] and Hill et al.[10] and their associates nearly simultaneously successfully used a different VAD as a bridge to transplantation. Portner and co-workers used the Novacor electrical assist device (LVAD) and Hill et al. used the Pierce-Donachy pneumatic one. We selected the Novacor device because it is placed within the abdominal wall, thereby achieving a heterotopic location that we believed was important for the avoidance of infection. Its electrical power source exiting remotely from the pump leaves open the possibility of total implantability of the system. A preclotted inflow conduit to the pump is placed within the left ventricular apex and tunnelled under the costal margin near the reflection of the diaphragm. Blood is thereby brought to the pusher-plate pump and directed through unidirectional biologic valves to the preclotted outflow conduit, which

TABLE 1. *Mechanical Circulatory Support Summary, University of Pittsburg (10/24/85–4/1/91)*

Status	ECMO	Biomedicus VAD	Jarvik TAH	Novacor LVAD	Totals
Implanted	10	9	20	23	62
Awaiting treatment	0	0	0	2	2
Died on device	2	3	3	4	12
Transplanted	8/10 (80%)	6/9 (67%)	17/20 (85%)	17/21 (81%)	48/60 (80%)
Discharged after treatment	5/8 (63%)	2/6 (33%)	9/17 (53%)	17/17 (100%)	33/48 (69%)
Alive at home	4	2	7	15	28

ECMO, extracorporeal membrane oxygenation; VAD, ventricular assist device; TAH, total artificial heart; LVAD, electrical assist device.

is anastomosed end-to-side to the ascending aorta. The pump itself is placed in a pocket in the left upper quadrant between the costal margin and the iliac crest in a plane between the posterior rectus sheath and the rectus abdominis muscle.

In contradistinction to the Pierce-Donachy pump, the Novacor was designed to support only the left ventricle. Its suitability for bridge to transplantation and possibly for permanent implantation depends on whether it can effectively sustain patients with biventricular failure. Although the Stanford program showed early promise for the LVAD,[11] other bridge-to-transplant programs suggested the need for biventricular support for most.[12]

To date 23 patients (21 men and 2 women) have received the Novacor device; 4 died while on the device; 2 await a donor heart (18 and 193 days of support); and 17 patients have been transplanted, and all have returned home. Our first two patients died with the effects of intraoperative bleeding, lack of peripheral vasomotor tone, and multi-organ failure. In addition the second patient sustained a large blood loss (15,710 mL) because of the amount of surgical dissection associated with bilateral internal mammary artery bypass grafts he had received 5 years previously. Both internal mammary arteries were transected during that dissection, and this contributed to some degree of cardiac failure. Two other youthful patients have died on the device with multi-organ failure that appeared to be imminently present before LVAD implantation. The 30-day survival of all patients transplanted compares with a 2% mortality rate for elective transplantation during this same period and to a 7% mortality rate for those requiring inotropic or intra-aortic balloon support before transplantation.

The indication for implantation was based on failure to maintain adequate renal, hepatic, or central nervous system function in the face of maximally tolerable medical support. All 23 patients had been on inotropic drugs for a median of 23 days (range, 2–71 days), and 20 patients had been on the intra-aortic balloon before the final decision to use the Novacor (median, 15 days; range, 1–38 days). In 14 patients the decision for implantation was primarily the presence of a cardiac index of less than 2.0 L/min/M^2 while on maximal support. In nine others with a cardiac index between 2.0 and 2.5 L/min/M^2, the decision for implantation was based primarily on clinical criteria, which included mental obtundation in five. Other factors included a rising serum creatinine, cool extremities, evidence of hepatic congestion, and a mixed venous oxygen saturation below 50%. Conversely we believed that the LVAD should not be used in a resuscitative setting, and therefore patients with sudden deterioration including renal and hepatic failure required a period of intense medical stabilization before LVAD implantation. Patients with renal (creatinine greater than 2.5 mg/dL or hepatic total bilirubin greater than 5 mg/dL dysfunction) were not candidates unless problems resolved without recurrence over 7 to 10 days.

Because of our unhappy experience with infection with the TAH, we searched diligently for sources of sepsis in candidates for the device and excluded candidates until infection was controlled.

The challenge of using the LVAD as a bridge to transplant was whether it was suitable for the majority of patients who would present with end-stage biventricular failure. Its effectiveness in the bridge-to-transplant setting in this regard also would have significant bearing on the appropriateness of its development as a permanent device. Of importance in this regard was our observation that all of those transplanted ultimately required only left univentricular support; 12 of 21 perioperative survivors required significant inotropic support of their right ventricle for more than 24 hours, accompanied by little or no change in their right ventricular ejection fraction. Right ventricular dysfunction resulting in poor filling of the Novacor prompted the use of mechanical right ventricular support (Biomedicus pump) in 5 of these 12. Although two of these patients died of multi-organ failure, three others required only temporary (2–4 days) right ventricular support and were converted to successful univentricular support for periods between 15 and 125 days before successful transplantation. Nine of the patients required only renal doses of inotropic agents by 24 hours after LVAD implant and demonstrated a progressive rise in right ventricular ejection fraction in the face of this reducing inotropic support. Unfortunately neither preimplant hemodynamics nor type of cardiomyopathy distinguished between those who required maximal *versus* those who required minimal support for the right ventricle after LVAD implant (Fig. 1). The near complete left ventricular afterload reduction (pulmonary capillary wedge pressure from 25 to 10 mm Hg) afforded by the LVAD also provided such dramatic right ventricular afterload reduction (mean pulmonary artery pressure from 39 to 25 mm Hg) to allow even a marginally functioning right ventricle to provide flow of blood to the LVAD.[13] A significant reduction in pre-LVAD transpulmonary gradient (11.7 to 8.1 mm Hg) and pulmonary vascular resistance (3.8 to 1.5 Wood Units) was determined when pre-LVAD measurements were compared with measurements made after orthotopic transplantation. In fact four patients had transpulmonary gradients (greater than 17 mm Hg) and pulmonary vascular resistances (greater than 5 Wood Units) before LVAD implantation, which would have significantly increased the risk for routine orthotopic transplantation.

Because preimplant hemodynamic evaluation and traditional measurements of right ventricular function did not predict who would require minimal *versus* maximal

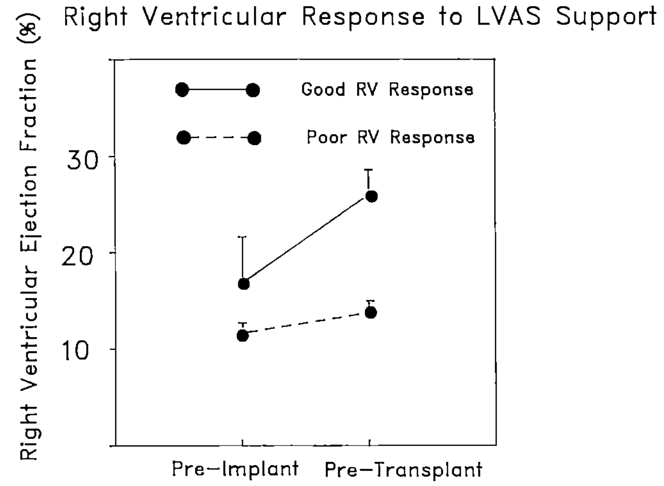

FIG. 1. Right ventricular ejection fraction for patients requiring RV support on LVAD (good response) *versus* maximal RV support (poor response).

right ventricular support during LVAD support (Table 2), we chose to study the pressure–volume response of the right ventricle by taking advantage of a unique liaison established with the School of Engineering at the University of Pittsburgh. They provided us with the expertise and technology to study the pressure–volume response of the right ventricle during LVAD support. Our initial reports were based on techniques of sonomicrometry measuring changes in segment length of the right ventricular free wall.[13,14] Analysis of biomechanical data showed a decrease in segmental myocardial power output, with no significant change in fractional fiber shortening in those patients requiring maximal right ventricular support. Analysis of pressure-segmental length loops suggested that the right ventricle functioned more normally during LVAD support compared with an isometric type of contraction, which occurred before LVAD implantation. Interpretation of data using this method was seriously impaired by the abnormal geometry and asymmetric motion of these grossly distended right ventricles. A more representative approximation of right ventricular volume was made by recording changes in right ventricular cross-sectional area obtained from intraoperative transesophageal echocardiograms and has facilitated estimates of right ventricular contractility, compliance, and energetics. Examination of pressure–area loops before and after LVAD implant (Fig. 2) demonstrated how the end-systolic point shifted to the left and down, accompanied by an increase in loop-area and approximation of work in those who required minimal inotropic support of the right ventricle. Conversely in patients who required mechanical RV support, the end-systolic point shifted minimally down but in a pathologic direction to the right. There also was seen a decrease in the loop area. More sophisticated analyses based on these techniques demonstrate that patients who had a requirement for right ventricular support during LVAD support had only minimal reduction in RV afterload or developed a reduction in right ventricular contractility. Currently we are attempting to use this technology to predict the response of the right ventricle before LVAD support.

Infection occurred during support in three patients, and all occurrences were related to the percutaneous drive line between the skin and the pump. In one patient this amounted to minor inflammation of the skin, but with positive *Staphylococcus aureus* cultures between days 29 and 40. This was controlled with local treatment and did not recur before transplantation on day 72. In the other two patients, deeper infections required intermittent intravenous antibiotics, local wound irrigation, and dressing changes. In one patient with a *Staphylococcus aureus* infection between day 40 and transplant on day 144, this had no sequelae after transplantation. Only one patient had a serious device-related infection. Cultures of the drive line exit site showed *S. aureus* and *Proteus mirabilis* 34 days after implant. Subsequent transplant on day 97 of support was followed 2 weeks later by a *Proteus* empyema and abdominal wall infection at the site of the previous LVAD pocket. This infection required pleural drainage,

TABLE 2. *Hemodynamic and Right Ventricular Function Data for Novacor LVAD Patients*

	Minimal RV Support		Maximal RV Support	
Hemodynamics	Pre-Novacor	Post-Novacor	Pre-Novacor	Post-Novacor
Heart rate (bpm)	100	90	118	110
Right atrial pressure (torr)	16.6	13.4	15.7	16.9
Pulmonary artery mean (torr)	39.8	23.7	37.6	26.6
PCWP (torr)	25.4	9.0	24.5	10.5
TPR (dynes-s/cm^5)	910	343	656	412
SVR (dynes-s/cm^5)	1281	1061	996	946
Cardiac index (L/min/m^2)	1.8	3.0	2.1	2.8
Inotrope equivalent (μg/kg/min)	25.4	15.9	32.4	52.5
RVEF (%)	16.8	26.0	11.6	13.7

LVAD, electrical assist device; RV, right ventricular; PCWP, pulmonary capillary wedge pressure; TPR, total pulmonary resistance; SVR, systemic vascular resistance; RVEF, right ventricular ejection fraction.

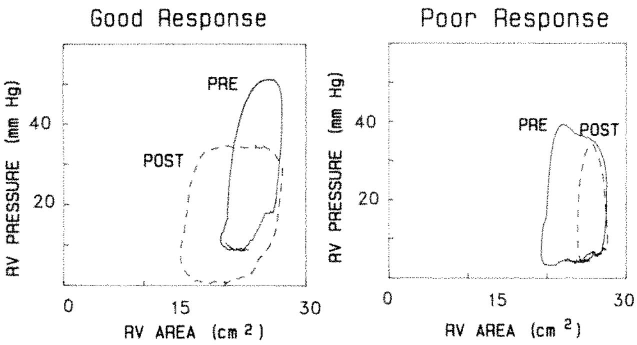

FIG. 2. Pressure-area loops for a patient requiring minimal RV support (left) and mechanical right ventricular support (right) on LVAD.

irrigation, and rotation of the rectus muscle flap. The reduced incidence of life-threatening infection in this series is attributed to the heterotopic location of the pump and avoidance of patients with suggested sepsis and imminent vital organ failure.

The LVAD thus appeared to answer our concerns relative to orthotopic devices and ethical concerns about using bridge-to-transplant technology less relevant to our goal of a permanent system. We believed that broad-based success with the LVAD would favor subsequent implications for appropriate programmatic directives in the development of future artificial hearts.

Recovery of patients sustained on the LVAS has been impressive, and the rehabilitation during the prolonged period of minimally traumatic support has contributed to the improved results of transplantation. All patients who were transplanted became ambulatory and developed an improved nutritional status. All were entered into a program of exercise that included stationary bicycling. Exercise performance between 6 and 16 weeks of support in six patients who were tested was greater than 5 METS with a peak oxygen consumption above 16 ml/min/kg. This was similar to that achieved by a group after transplantation tested at a similar time.

In view of the health of these patients and recognizing the increased time spent on support, which averaged 80 days in 1990, as well as the reliability of the device, permission was sought and obtained from the Food & Drug Administration for selected patients on the Novacor to be housed in a nearby minimal care home while awaiting transplantation.[15] Issues relating to electrical safety, emergency power, patient transport to and from the hospital, physician, nursing and biomedical engineering responsibilities, and patient and family education and training were addressed not only for the bridge patients but also with anticipation of a chronic LVAS trial (Fig. 3). Two patients, a 15-year-old boy and a 37-year-old woman, spent 5 and 18 consecutive days, respectively, in this facility immediately before transplantation.

Our initial predictions that the waiting period for pharmacologically or mechanically supported candidates would increase during this era were realized as the waiting period before device implantation averaged 30 days, and the average waiting period for a donor heart while on a mechanical circulatory support device increased from 10 days in 1987 to 80 days in 1990 (Fig. 4). The increased need for personnel to manage these device patients, which at times simultaneously included two in hospital and one off-site, was met through the development of a strong core of 14 part-time and 4 full-time biomedical engineers. As

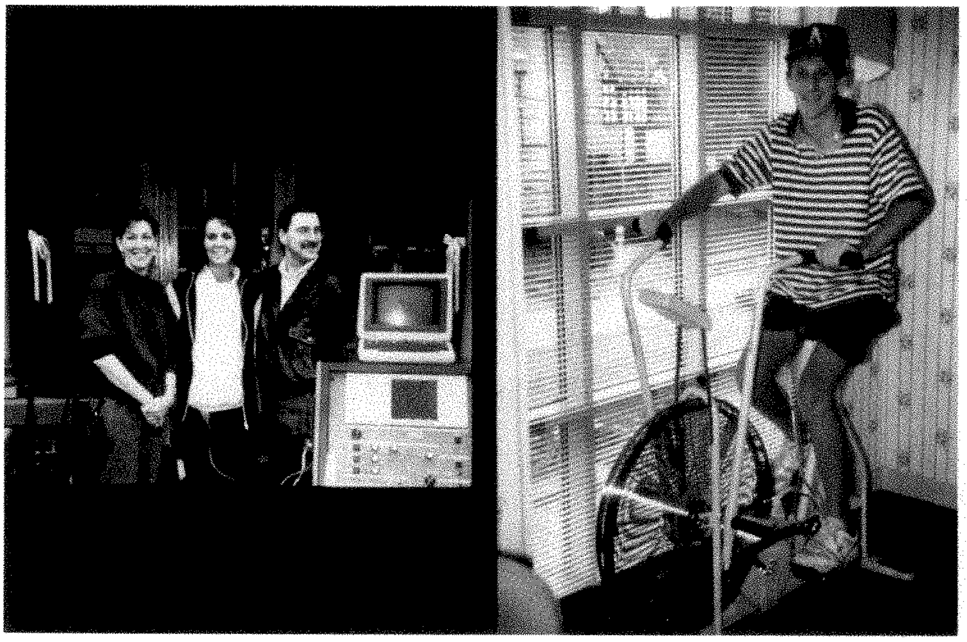

FIG. 3. Patients awaiting transplant in outpatient facility.

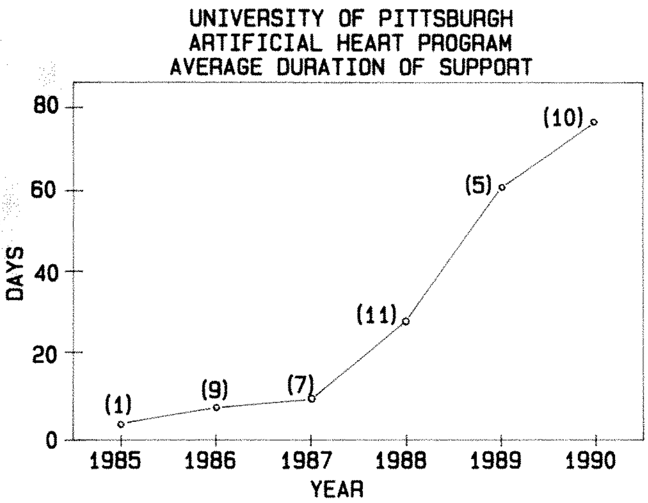

FIG. 4. Average mechanical support time for patients awaiting transplant at the University of Pittsburgh Artificial Heart Program. Numbers in parentheses indicate the number of patients supported per year.

the clinical need was met, we developed a more broad-based program that fostered 2 Masters theses, 2 Ph.D. theses, 3 fellowships, and 6 NIH submissions (3 funded, 3 pending).

Through the past decade of transplantation, strategies for dealing with severely ill candidates who have waited for progressively longer times for donor organs have provided lessons that we now hope to apply to future chronic mechanical circulatory support technology. The challenges provided in the early and total artificial heart era were met through a programmatic approach to the problems of patient selection, infection, quality of life, and the adaptation of heterotopic univentricular technology in the face of biventricular failure. The experience of 1521 patient/device-days accrued during the past 5 years has prepared us enthusiastically for our next challenge, an NIH-sponsored chronic implantable LVAD trial.

Acknowledgments

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References

1. Bahnson HT, Starzl TE, Hakala TR, et al. Development and organization of a multiple organ transplantation program. Ann Surg 1986; 203(6):620–625.
2. Hardesty RL, Griffith BP, Trento A, et al. Mortally ill patients and excellent survival following cardiac transplantation. Ann Thorac Surg 1986; 41:126–129.
3. Reemtsma K, Drusin R, Edie R, et al. Cardiac transplantation for patients requiring mechanical circulatory support. N Engl J Med 1978; 298:670.
4. DeVries WC. The permanent artificial heart. JAMA 1988; 259(6): 849–859.
5. Levinson MM, Smith RG, Cork RC, et al. Three recent cases of total artificial heart prior to transplantation. Heart Transplant 1984; 5:170–175.
6. Griffith BP. Interim use of the Jarvik-7 artificial heart: lessons learned at Presbyterian-University Hospital of Pittsburgh. Ann Thorac Surg 1989; 47:158–166.
7. Griffith BP, Hardesty RL, Kormos RL, et al. Temporary use of the Jarvik-7 total artificial heart prior to transplantation. N Engl J Med 1987; 316:130–134.
8. Kaplan SS, Basford RE, Kormos RL, et al. Biomaterial associated impairment of local neutrophil function. Trans Am Soc Artif Intern Organs 1990; 36:M172–M175.
9. Portner PM, Oyer PE, Jassawalla JS, et al. A totally implantable ventricular assist device for end-stage heart disease. *In* Unger F, ed. Assisted Circulation 2. Berlin: Springer-Verlag, 1984, pp 115–141.
10. Hill JD, Farrar DJ, Hershon JJ, et al. Use of a prosthetic ventricle as a bridge to cardiac transplantation for post-infarction cardiogenic shock. N Engl J Med 1986; 314:626.
11. Starnes VA, Oyer PE, Portner PM, et al. Isolated left ventricular assist as bridge to cardiac transplantation. J Thorac Cardiovasc Surg 1988; 96:62–71.
12. Farrar DJ, Hill JD, Gray LA, et al. Heterotopic prosthetic ventricles as a bridge to cardiac transplantation: a multicenter study in 29 patients. N Engl J Med 1988; 318:333–340.
13. Kormos RL, Borovetz HS, Gasior T, et al. Experience with univentricular support in mortally ill cardiac transplant candidates. Ann Thorac Surg 1990; 49:261–272.
14. Kormos RL, Gasior T, Antaki JF, et al. Evaluation of right ventricular function during clinical left ventricular assistance. Trans Am Soc Artif Intern Organs 1989; 35:547–550.
15. Kormos RL, Borovetz HS, Pristas JM, et al. Out-of-hospital facility for the Novacor bridge to transplant patient: the Pittsburgh Family House experience. Abstract of the American Society for Artificial Organs 37th Annual Meeting, April 25–27, 1991.

DISCUSSION

DR. W. S. PIERCE (Hershey, Pennsylvania): In 1974 Dr. John Waldhausen and I described the results of animal studies using a paracorporeal ventricular assist pump before a meeting of this Society. Two years later, that device was successfully used to support the circulation of a patient who had undergone mitral valve surgery and coronary bypass grafting.

There have now been over 150 clinical applications of this pump, both in the United States and abroad, after open-heart operations, allowing time for recovery of the natural heart. In 1985 the pump was successfully used as a bridge for cardiac transplantation. To date there have been 134 such applications. In 33 instances, the device was used for isolated left ventricular support with 20 survivors. In 101 instances, two such devices were employed, providing biventricular support. There have been 53 survivors in this group.

I certainly enjoyed the presentation of Dr. Kormos and his colleagues. Rarely does one institution have such a significant experience with a variety of devices. We generally agree that most patients who require a bridge to cardiac transplantation can be effectively supported with a single left ventricular assist pump, but there also has been an excellent experience with the use of two pneumatic pumps supporting the entire circulation.

I have two questions for Dr. Kormos. First I would like to ask whether he and his colleagues believe there is any role for the total artificial heart in the bridge to transplantation. And, if so, what patients would be considered appropriate candidates?

Secondly I would like to ask Dr. Kormos, what preoperative and operative criteria are presently used to determine if a patient's circulation can be supported with a single left ventricular assist pump, or if biventricular assistance will be required?

Dr. G. Pennington (St. Louis, Missouri): I think the single most important contribution has been the demonstration that temporary circulatory support can lead to a successful transplantation rate that is similar to or better than that of patients not requiring circulatory support.

Furthermore it demonstrates in a large majority of the patients, that even with biventricular failure, a single left ventricular assist device can provide satisfactory support.

Dr. Kormos alluded to the fact that permanent implantation of these devices was to be supported by an NHLBI contract in the future.

These studies are to begin within the next year, in which a Novacor device will be used with an external belt to transmit energy by inductive coupling across the skin to an internal battery system and then to the same pump system and cannula system that he has already demonstrated.

We believe that this system will provide patients with a tether-free environment in which they may resume near-normal activities. We anticipate that patients will be able to remove the belt for approximately 45 minutes for showering or other activities, and that this device will provide patients with a more or less normal existence as a permanent system.

I would submit to the Society that the previous decade has demonstrated the efficacy of these devices as temporary support systems, and that the current decade will demonstrate the effectiveness of these devices as permanent, totally implantable systems.

I have two questions for Dr. Kormos. Number one: How will he choose those patients with biventricular failure for permanent support with a single left ventricular assist device?

Is there a role for a total artificial heart in the permanently supported patients?

And, finally, even though the thromboembolic rate was low in the series, it was not insignificant. Can this be improved on?

Dr. Robert L. Kormos (Closing discussion): First of all I would like to collectively thank Drs. Pierce and Pennington. As they and everyone realize, we are relative newcomers to the field of mechanical circulatory support following on the heels of their pioneering work. Their thoughts, guidance, and innovative approach to this very ill class of patients has spurred us on to achieve the results that I have been able to present to you today.

First of all Dr. Pierce and Dr. Pennington, as far of the role of the total artificial heart in the acute setting, I think at present we have relegated its role to two situations.

One of the things that clearly determines the degree and manner of development of biventricular failure is the presence of ischemia. Biventricular failure in the setting of the chronically dilated and accommodated heart, such as I was showing you today, is a different setting than the type of situation that occurs with acute ischemia, where the contribution of the septum and thus performance of both right and left ventricles can be affected. The discrepancy in the role of the septum that one sees in the literature, where we see reported a high incidence of right heart failure developing in the presence of left ventricular support, may reflect the role of ischemia.

Therefore in situations of acute infarction, where both right and left ventricles are at risk, there may be a role of the total artificial heart.

Second of all in situations of acute ischemic rupture of the univentricular septum, it is very difficult, if not impossible, to support patients either univentricularly or biventricularly because of the blood that shunts between the ventricles, so this may be another possible role for the artificial heart.

As far as the preoperative and intraoperative assessment of right ventricular function before left ventricular assist, we currently are looking at the amount of inotropic support that is required for patients as they are weaned onto the left ventricular device in the operating room. At the time of chest closure, it is very obvious to us using some of the echocardiographic methods that are shown to you today whether or not right ventricular failure is developing, and I think that using these methods we may be able to answer to the second question that Dr. Pennington posed, which is how to decide who needs the total artificial heart for chronic heart failure. At this time I do not think we can say whether or not we can make that decision preoperatively. We have no clear-cut answers at this point.

As far as the situation of stroke and thromboembolism, we have had two patients with a chronic persistent neurologic deficit and four others with temporary, very short neurologic deficits from which they totally recovered.

We think that many of these are related to potential valve design problems that are being dealt with, but also hemodynamic and filling issues, which we are trying to address. If right heart failure is too severe or in situations where right ventricular support is required, the flow to the left side may be limited, thus producing a setting where the pump is filling incompletely.

Pulsatile Reperfusion After Cardiac Arrest Improves Neurologic Outcome

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Cardiopulmonary bypass (CPB) using nonpulsatile flow (NPF) is advocated for refractory cardiac arrest. This study examined cerebral outcome after resuscitation with pulsatile flow (PF) *versus* NPF. Dogs arrested for 12.5 minute were reperfused with NPF ($n = 11$) using roller pump CPB or PF ($n = 11$) using mechanical biventricular cardiac massage. Pump flows were similar between groups; however early arterial pressures were greater during PF *versus* NPF, $^{*}p < 0.05$. Circulatory support was weaned at 60 minutes' reperfusion. Neurologic recovery of survivors ($n = 16$) was significantly better after PF *versus* NPF, $^{*}p = 0.01$. The presence of brain lesions on magnetic resonance images did not significantly differ between groups at 7 days. Brains then were removed and regions examined for ischemic changes. Loss of CA1 pyramidal neurons was more severe after NPF *versus* PF, $^{\dagger}p = 0.009$. Ischemic changes were more frequent after NPF in the caudate nucleus ($^{\dagger}p = 0.009$) and watershed regions of the cerebral cortex ($^{\dagger}p = 0.062$), compared with PF. These results demonstrate that PF improves cerebral resuscitation when treating cardiac arrest with mechanical circulatory support (* = MANOVA with repeated measures, † = categorical data analysis).

CEREBRAL ISCHEMIA RESULTS in the tragic loss of neurologic function if not adequately reversed in a timely fashion. This circumstance occurs in the setting of cardiac arrest, the most common cause of clinical death. Ironically therapies that improve cardiac resuscitation have raised concern about the increased potential for successfully resuscitating severely brain-damaged patients. This dilemma seems inevitable in view of the brain's limited tolerance to ischemia. Brief periods of circulatory arrest (5–10 minutes) can irreversibly damage the brain.[1-5] In contrast the heart can tolerate significantly longer periods of ischemia and subsequently sustain life.[6] Therefore preserving cerebral function has become a major challenge for investigators attempting to improve resuscitation from cardiac arrest.

Recent attention has been directed toward mechanical circulatory support as an adjunctive treatment for refractory cardiac arrest. Previous experience found these devices effective for recovering cardiac function.[7] New advances in cannulation techniques make institution of blood pumps possible during cardiac arrest.[8-11] Although these support systems vary somewhat, most use a standard cardiopulmonary bypass (CPB) circuit with either roller or centrifugal pumps. Both of these devices provide resuscitative support with nonpulsatile flow. Certain characteristics of pulsatile flow, however, may be critical for effective resuscitation. Hydraulic forces generated by pulsatile flow are thought to be required for maintaining the patency of capillary beds.[12-14] In a similar manner, the cerebral microcirculation may depend on "critical opening pressures" during reperfusion. Although cardiac arrest represents the most common clinical condition causing global cerebral ischemia, prior investigations have not compared the effects of pulsatile and nonpulsatile flow on neurologic outcome. The purpose of this study, therefore, was to assess cerebral recovery after cardiac arrest and subsequent resuscitation using either pulsatile or nonpulsatile reperfusion.

Methods

The study protocol was approved by the Animal Care and Use Committee at Duke University Medical Center and performed in accordance to the "Guide for the Care and Use of Laboratory Animals" prepared by the National Research Council of the NIH (NIH Publication No. 86-

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23, Revised 1985). Twenty-two young (12–16-month-old) conditioned coon hounds (23 ± 1 kg) from the same breeding colony were chosen for this study. Only dogs in good physical health, tested negative for heart worms, and with no evidence of respiratory disease or infection were selected for experimentation.

The experiment modeled cardiac arrest by electrically inducing ventricular fibrillation (VF). Mechanical circulatory support was employed for resuscitation after 12.5 minutes of VF. Animals were separated into two groups for either pulsatile or nonpulsatile circulatory support.

Nonpulsatile flow (NPF) was generated from a standard cardiopulmonary bypass system (Fig. 1). The system used two venous return cannulae positioned in the superior and inferior vena cavae, a Cobe membrane oxygenator, and a calibrated roller pump that delivered nonpulsatile arterial return through a femoral cannula. Pulsatile flow (PF) was produced by a method termed direct mechanical ventricular actuation (DMVA) (Fig. 2). Direct mechanical ventricular actuation is achieved by a pneumatic device that alternately compresses and dilates the ventricular chambers and has been described in detail elsewhere.[15–20] The device can best be understood as a mechanical method of internal cardiac massage. Direct mechanical ventricular actuation application during VF can reliably generate pulsatile flow that is virtually identical to the normal physiologic state.

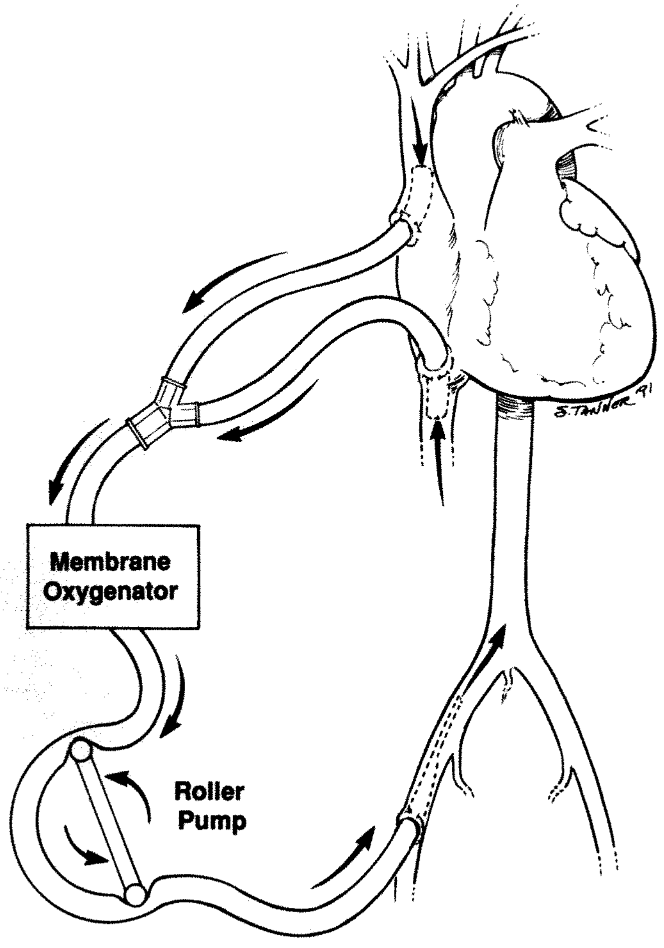

FIG. 1. Schematic diagram of cardiopulmonary bypass circuit used to generate NPF. Bicaval cannulation was used to ensure maximal venous return.

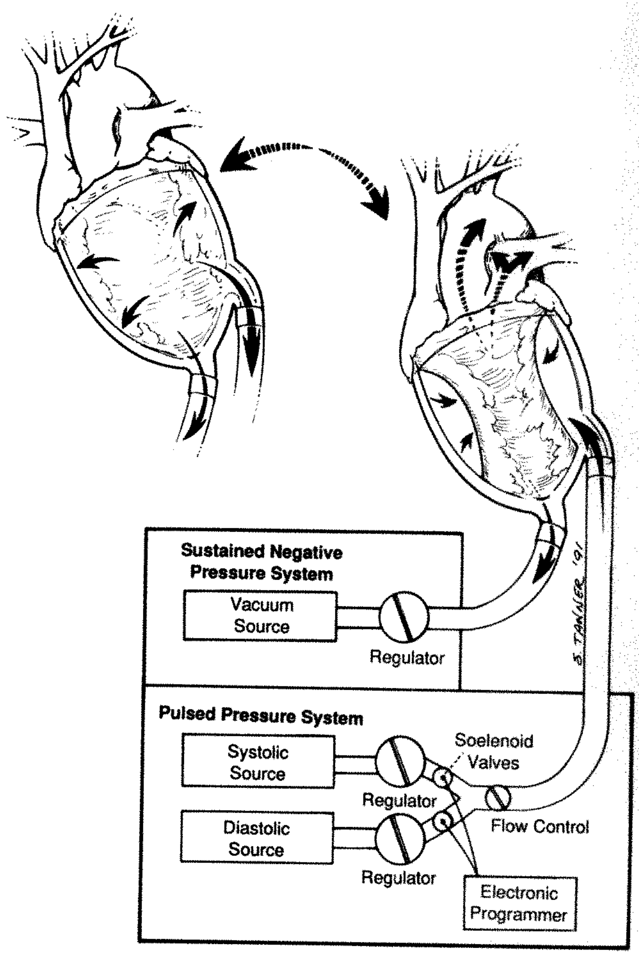

FIG. 2. Schematic of DMVA used to generate PF. Regulated by a pneumatic drive system, an assistor cup actuates the ventricles into systolic and diastolic configurations.

Animal Preparation

Dogs were fasted the evening before experimentation with access only to water. An intravenous (I.V.) line was placed for infusion of Lactated Ringer's (LR) solution on the morning of the study. Sodium pentobarbital (I.V.) was administered slowly until the corneal reflex was ablated; this reflex was subsequently monitored until completion of the operative procedure. The animals were intubated, given procaine penicillin G (600,000 units) and dihydrostreptomycin sulfate (750 mg) (I.M.) and then prepared for a lateral thoractomy using aseptic technique. An electronic thermistor probe was positioned in the rectum for measuring core temperature. Respiratory support was begun with a pressure-regulated ventilator (InterMed Bear 1) before any operative procedures.

All surgery was performed in a standard sterile fashion. Polyethylene catheters positioned in the femoral artery allowed monitoring of arterial blood pressures and blood gases. The opposite femoral artery was exposed for arterial cannulation in those animals selected to receive CPB. Animals receiving DMVA had a Swan-Ganz catheter advanced to the pulmonary artery for calculating blood flow during resuscitation. Lateral thoracotomies were then made through the right or left chest for CPB or DMVA, respectively. After confirming surgical hemostasis, heparin (100 U/kg, I.V.) was administered 5 minutes before cardiac arrest in both groups. Ventricular fibrillation was induced by low-voltage AC current.

During cardiac arrest an arterial return cannula was advanced into the femoral artery of dogs receiving CPB. The right atrium was then exposed by pericardiotomy and two venous cannulas positioned with their tips in the proximal superior and inferior vena cavae. After 10 minutes of arrest, the pericardium was widely opened in both groups of animals. At 12 minutes of arrest, an appropriately sized assistor cup was positioned over the hearts of dogs receiving DMVA.

Resuscitation Protocol

Circulatory support was initiated after 12.5 minutes of VF arrest in both groups. Cardiopulmonary bypass flow rates were advanced to 100 ml/kg/min during the first 2 minutes of support. Flow rates were constantly adjusted thereafter, to match maximal venous return. Direct mechanical ventricular actuation compression rates were begun at 60 cycles/minute with actuating forces adjusted to the lowest degree allowing full compression and dilatation of the ventricles. Systolic durations remained at 50% throughout DMVA support. Cycle rates, as well as systolic and diastolic forces, were gradually increased during the first 2 minutes of support to 120/minute, +125 mm Hg, and −110 mm Hg, respectively. Hearts were defibrillated after 15 minutes of reperfusion in both groups. Fibrillation refractory to three successive countershocks (10, 20, and 30 J) was treated by similar defibrillation attempts every 15 minutes.

Total circulatory support was continued for a full hour in both groups. Arterial blood pressures, pump flows, serum electrolytes, and arterial blood gases were recorded at 5, 15, 30, 45, and 60 minutes of reperfusion. All animals were given sodium bicarbonate (2 mEq/kg) during the first 5 minutes of reperfusion. Thereafter sodium bicarbonate, potassium chloride, and calcium chloride were administered every 15 minutes as needed to correct arterial blood gases and serum electrolytes. Respiratory settings and membrane oxygenator gas flows were adjusted to keep arterial PO_2 between 150 and 250 mm Hg and PCO_2 between 30 and 45 mm Hg.

After 60 minutes of reperfusion, circulatory support was discontinued. In animals treated by CPB, the entire pump volume (1 L of LR prime) was returned to the circulation over 15 minutes. One unit of fresh whole blood was then given over 45 minutes, followed by LR at 100 mL/hour for 12 hours. Animals supported by DMVA received 1 L LR during support. Thereafter fluid administration was decreased to 100 mL/hour for 12 hours after removal of the device. Hemostasis was obtained by electrocautery, followed by application of Thrombin spray (Thrombostat 20,000 units) to all surgical incisions. A size 20 French chest tube was placed and the chest was closed.

Arterial pressures and blood gases were monitored for 1 hour after support. The catheters then were removed and the groin incisions closed. Ventilatory support was maintained for 3 hours after cessation of circulatory support. Animals then were placed on 50% oxygen and transferred to their cages. Four hours after resuscitation, 10 mL 50% dextrose and 25 mg flunixin (a nonsteroidal anti-inflammatory agent) were administered I.V. Animals were extubated 6 hours after resuscitation. Oxygen supplementation was continued for the following 12 hours. Analgesia was insured by administering morphine sulfate (0.5 mg/kg I.V.) immediately after circulatory support with two subsequent doses of 0.25 mg/kg at 6 and 12 hours (after the neurologic assessments, below). Intravenous fluids were decreased to 50 mL/hour 16 hours after resuscitation and discontinued once animals began taking oral fluids. Animals were fed and watered by hand two times per day until daily requirements were taken spontaneously. Tube and hand feeding were continued if voluntary intake was inadequate.

Assessment of Neurologic Function

Neurologic function was based on two scoring systems. One rated neurologic recovery during the 7-day postoperative period. The other scoring system measured persistent neurologic deficits at the end of the recovery period.

Neurologic recovery scores were based on those used by previous investigators.[8,21–23] Only the neurologic activities that could be objectively assessed were chosen for monitoring recovery. Scores were assigned to each of 12 categories: corneal reflex (present = 1, absent = 0); respiratory effort (present = 1, absent = 0); pupillary response (normal = 3, sluggish = 2, pinpoint = 1, fixed & dilated = 0); swallow (present = 1, absent = 0); visual perception (gross objects = 2, light only = 1, none = 0); pain response (present = 1, absent = 0); touch response (present = 1, absent = 0); voice response (present = 1, absent = 0); food intake (without assistance = 2, hand feeding = 1, none = 0); sternal recumbency (yes = 1, no = 0); standing (yes = 1, no = 1); and walking (without falling = 2, with falls = 1, unable = 0). Neurologic function was assessed at 3, 6, 12, 18, and every 24 hours thereafter by a veterinarian unaware of the animal's resuscitative therapy.

A separate neurologic assessment determined persistent neurologic deficits after 7 days of recovery. Animals were assigned one point for unremitting visual impairments, gait defects, ambulatory incapacity, or indifference to food on day 7, as well as for any seizure activity during the entire recovery period. Each animal received a deficit score based on the presence or absence of these five abnormalities (range, 0[normal] to 5[severe deficit]).

Magnetic Resonance Imaging

After 7 days of recovery, animals were anesthetized with pentobarbital for magnetic resonance imaging (MRI). Brain imaging was performed in a 1.5-Tesla clinical imager (GE Signa) using a quadrature head coil. Coronal T2-weighted 5-mm-thick images were obtained at 7.5-mm intervals. Sequence parameters for imaging included: a TR of 2800 msec, TE of 30 and 80 msec; number of excitations = 0.75; and a field view of 16 cm.

Comparisons were made between the two echoes to separate normal areas of high signal intensity (*i.e.*, cerebrospinal fluid) from brain lesions.

All images were interpreted by a radiologist blinded to the animals' treatment protocol.

Brain Histopathology

The day after MRI assessments (8 days after resuscitation), animals were killed and their brains placed in 10% formalin for at least 2 weeks. The brains then were sectioned coronally and inspected for gross lesions. Regions most frequently injured on MRI images were examined histopathologically. Representative blocks of the hippocampus, caudate nucleus, cerebellum, and watershed areas of the frontal and parietal cortex were taken from each hemisphere, placed in 20% sucrose/10% formalin, and cut on a freezing microtome. Representative sections were mounted on gel-coated slides and stained for neurons with cresyl violet.

Hippocampal damage was graded by scoring pyramidal cell loss within the CA1 region. Those hippocampi with no identifiable injury were assigned a score of 1 (normal). Those with complete absence of CA1 pyramidal cells were assigned a score of 5 (severe). Those hippocampi that did not fall into these two extremes were categorized according to their overall relative appearances: scant pyramidal cell loss = 2, approximately 50% pyramidal cell loss = 3, and only scattered pyramidal cells remaining = 4. Scores from the left and right hippocampus were averaged to provide a single score for each animal.

The caudate nucleus was examined for evidence of ischemic injury as evidenced by microinfarction or diffuse neuronal loss. Abnormal findings were categorized into three groups: absent, unilateral, or bilateral.

The frontal and parietal watershed regions of brains were examined for evidence of histologic damage (microinfarction or diffuse cell loss). Results then were categorized for the two groups: injury found in one or more watershed regions *versus* absence of ischemic changes in all examined regions.

The cerebellum was examined for microinfarction or selective Purkinje cell loss.

All brain regions of surviving animals were histologically evaluated. One tissue block was unavailable for each region. Therefore each region was examined in 15 rather than in 16 cases. Tissue examinations and gradings were performed by a neuropathologist who was blinded to the experimental groups.

Statistical Analysis

Univariate analysis and between-group comparisons (PF *vs.* NPF) were performed using SAS statistical software (SAS Institute Inc., Cary, NC). Pre-arrest parameters known to affect neurologic outcome (temperature, pH, $PC0_2$, PO_2, and anesthetic dosage) were compared between groups using a nonpaired Student's t test. Variables known to affect neurologic outcome during resuscitative support (blood flow, mean arterial pressure, pulse pressure, temperature, pH, PCO_2, and PO_2) also were tested for between-group differences. Group (time by group effect) and within-group (within-group effect) effects were determined using multivariate analysis of variance with repeated measures over time (MANOVA). In addition overall between-group differences were tested using area under the curve analysis (group effect).

Experimental measures of cerebral recovery then were assessed. Serial neurologic scores were compared between groups using MANOVA. All measures of outcome that were categorized (MRI lesions, neurologic deficit scores, histologic grading, and occurrence of regional ischemic damage) were assessed by comparing row mean scores using a chi-squared statistic. Comparisons were considered statistically significant at alpha levels less than 0.05.

Results

Survival

Sixteen animals (73%) survived for the full 1-week observation period. All deaths occurred during the initial 18 hours of recovery. There was no significant difference in

TABLE 1. *Parameters Measured Immediately Before Cardiac Arrest (mean ± SEM)*

Parameter	PF	NPF	p
Temperature (°C)*	35.9 ± 0.2	35.4 ± 0.2	0.09
pH	7.41 ± 0.02	7.43 ± 0.01	0.50
pCO_2 (mmHg)	31 ± 2	28 ± 1	0.41
pO_2 (mmHg)	247 ± 19	208 ± 18	0.18
Pentobarbital (mg/kg)	31.2 ± 1.1	33.0 ± 1.7	0.30

* Temperatures tended to be 0.5 C lower in animals that received NPF *versus* PF. (PF *versus* NPF, nonpaired Student's t test).

NPF, nonpulsatile flow; PF, pulsatile flow.

mortality after PF (2/11) *versus* NPF (4/11), p = 0.31. One animal (PF group) succumbed to respiratory failure because of underlying pulmonary disease. The remaining deaths were of undetermined origin. Necropsies found no evidence of pneumothorax or hemothorax, pulmonary edema, or internal bleeding. Only results from the 16 surviving animals were analyzed in this study.

Control and Resuscitation Conditions

Prearrest variables that may affect neurologic outcome were kept similar in both groups (Table 1). Both groups of animals had mild hypothermia created by open thoracotomy. Core temperatures were slightly lower in animals before NPF compared with PF, but this difference was not statistically significant. Blood gas values exhibited little between-group disparity and mean sodium pentobarbital doses differed by less that 2 mg/kg. All animals were normotensive with a stable sinus rhythm before induction of ventricular fibrillation.

Variables monitored during the period of resuscitative circulatory support are summarized in Table 2. Blood flow was calculated by thermodilution and a calibrated roller pump during PF and NPF, respectively. Flows were entirely dependent on the rate of maximal venous return in both groups. Calculated flows were not significantly different between groups. Pilot studies and prior investigations using radiolabeled microspheres under similar experimental conditions found roller pump values underestimate actual flow by approximately 25% compared with thermodilution. Therefore actual flows were more closely matched between groups than measured values suggest. Accordingly calculated flows were compared to illustrate venous return trends for each group.

Despite similar flows PF initially generated greater mean arterial pressures (MAP) than did NPF. Resuscitative pressures significantly differed at 5 and 15 minutes of reperfusion. However MAP disparities diminished throughout reperfusion and did not significantly differ between groups after 15 minutes. Pulse pressures remained constant during PF and contributed to the relatively early elevations in MAP. Nonpulsatile flow exhibited small pulsations due to roller pump oscillations, which were periodically intensified by effective cardiac contractions. However PF pulse pressures consistently exceeded those during NPF by fivefold to tenfold.

Arterial blood gases were similar between groups, with the exception of pH values. Reperfusion with NPF resulted in rapid normalization of arterial pH, whereas a more gradual return occurred during PF. Thus mean pH values were significantly lower during the initial 15 minutes of PF compared with NPF. Thereafter pH remained similar between groups. Between-group differences in $P0_2$ were inconsequential, as they remained between 100 and 250 mm Hg in both groups.

Core temperatures also behaved differently between groups. Although mean values were identical at 5 minutes' reperfusion, core temperatures subsequently increased

TABLE 2. *Reperfusion Parameters During 1 Hour of Resuscitative Support with Either PF or NPF Flow (mean ± SEM)*

		ReperfusionTime (min)					
Parameter	Method	5	15	30	45	60	p
Flow (ml/kg/min)*	PF	89 ± 11	148 ± 15	159 ± 20	161 ± 18	146 ± 15	0.059
	NPF	110 ± 4	116 ± 7	108 ± 5	102 ± 2	100 ± 3	
MAP (mmHg)	PF	125 ± 6	109 ± 3	101 ± 6	100 ± 7	99 ± 6	0.033
	NPF	64 ± 7†	69 ± 7†	74 ± 8	79 ± 11	90 ± 11	
Pulse pressure (mmHg)	PF	75 ± 2	83 ± 8	79 ± 11	87 ± 8	79 ± 11	0.018
	NPF	8 ± 3	9 ± 3	14 ± 4	10 ± 2	18 ± 10	
Temperature (°C)	PF	35.5 ± 0.2	35.5 ± 0.2	35.5 ± 0.02	35.3 ± 0.3	35.4 ± 0.3	0.022
	NPF	35.3 ± 0.1	36.0 ± 0.1†	36.4 ± 0.3†	36.4 ± 0.2†	36.5 ± 0.2†	
pH	PF	7.17 ± 0.02	7.25 ± 0.02	7.36 ± 0.02	7.38 ± 0.01	7.39 ± 0.02	0.072
	NPF	7.36 ± 0.04†	7.37 ± 0.04†	7.43 ± 0.02	7.38 ± 0.01	7.35 ± 0.02	
pCO_2 (mmHg)	PF	46 ± 3	41 ± 2	36 ± 2	34 ± 1	36 ± 2	0.162
	NPF	40 ± 4	34 ± 3	30 ± 2	32 ± 1	38 ± 4	
pO_2 (mmHg)	PF	185 ± 24	165 ± 25	160 ± 20	136 ± 19	123 ± 15	0.072
	NPF	196 ± 19	223 ± 29	194 ± 26	188 ± 19	203 ± 23	

* Flow was calculated by thermodilution (PF) and a calibrated roller pump (NPF).

† $p < 0.05$ between groups for within group rate differences (MANOVA).

PF, pulsatile flow; NPF, nonpulsatile flow; MAP, mean arterial pressure.

during NPF. This small increase in temperature was statistically significant during the latter 45 minutes of reperfusion. At completion of circulatory support, mean temperature was 1 C higher in the NPF group.

Hemodilution was controlled by fluid administration. Animals in each group received equal volumes of crystalloid during resuscitation. Furthermore 1 unit fresh whole blood was given to animals after NPF to compensate for any hemolysis. Despite these factors mean hematocrit was somewhat decreased at 1 week after NPF (33.4 ± 1.8%) *versus* PF (39.4 ± 1.4%), $p = 0.058$.

Neurologic Outcome

Neurologic function returned significantly more rapidly during the first 24 hours in animals with PF, as evidenced by serial neurologic scores (Fig. 3). Although mean scores remained higher in the PF group than the NPF group, differences were not statistically different after 24 hours. This trend toward persistent neurologic discordance between the two groups was reflected in the neurologic deficit scores at 7 days (Fig. 4). Compared with four animals after NPF, only two animals treated by PF had an abnormal deficit score. Although the median deficit score was greater after NPF (1) than PF (0), the difference did not reach statistical significance. Furthermore there were no significant between-group differences with respect to individual neurologic abnormalities used in determining the neurologic deficit categories.

Magnetic Resonance Imaging

Magnetic resonance imaging detected gross brain lesions on T2-weighted scans (Fig. 5). Lesions were present in 4 of 13 brains scanned. The absence of lesions in each group (5/6 with PF *vs.* 4/7 with NPF), did not significantly differ (Fig. 6). Lesions numbered from two to nine per brain and occurred most commonly in the region of the caudate. Other areas damaged, in order of lesion incidence, included: the hippocampus, pons/midbrain junction, and cerebellum, with isolated lesions in the brainstem and frontal cerebral cortex. In contrast visual inspection of formalin-fixed coronal brain sections found no gross evidence of lesions. Only microscopic examination was able to identify areas of ischemic damage.

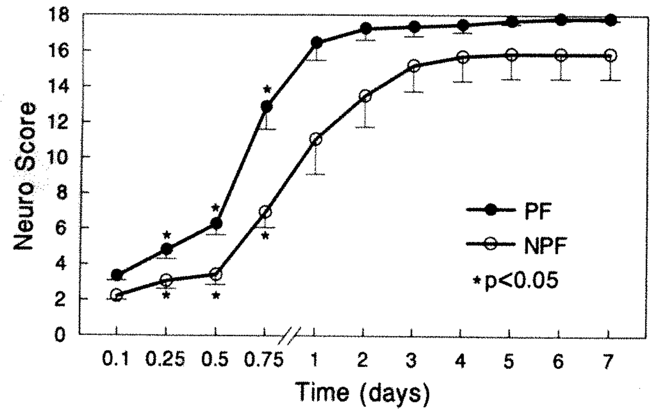

FIG. 3. Mean neurologic scores ± SEM after resuscitation with PF or NPF. The rate of neurologic recovery was significantly greater after PF. $p = 0.018$, group effect; *indicates significant differences within group recovery rates (within group effect); neuro, neurologic.

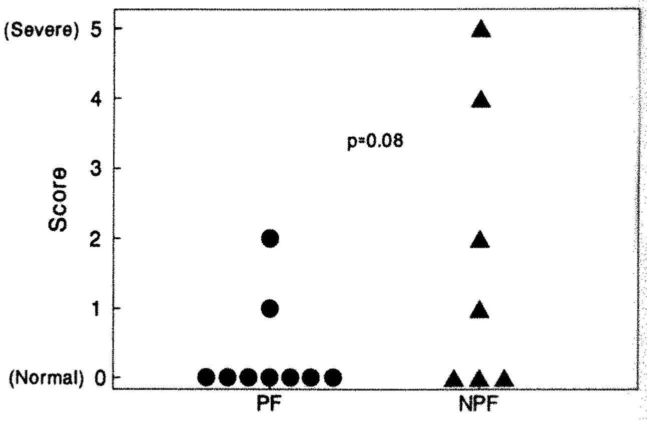

FIG. 4. Neurologic deficit scores after 7 days of recovery from cardiac arrest. Animals that received NFP showed a trend toward more severe deficits than those that received PF. Each symbol indicates an individual dog. (PF *versus* NPF, categorical analysis).

Brain Histopathology

Hippocampal injury was found in 10 of 15 animals, three of eight with PF *versus* seven of seven NPF, as determined by grading CA1 pyramidal neuronal loss (Fig. 7). Left and right hippocampal injury scores were averaged; however grades were identical in 11 of 15 brains. No areas of microinfarction were noted in any of the hippocampal sections. The extent of damage differed significantly between groups, with more severe injuries occurring in animals treated with NPF (Fig. 8).

The occurrence of caudate injury also was significantly greater after NPF than after PF (Fig. 9). Injury was evident in 1 of 8 brains from the PF group and 5 of 7 after NPF. One brain from the NPF group had diffuse neuronal loss and gliosis; the other four brains with damage had one or more microinfarcts.

Relatively little damage was found in the cortical watershed areas. There was no laminar necrosis or detectable selective neuronal loss, and only occasional microinfarcts were found. These were present only in the NPF group (Fig. 10), but between-group differences were not statistically significant.

No histopathologic changes were found in the cerebellar cortex.

Discussion

Experimental observations suggest that cerebral function may be better preserved by PF than by

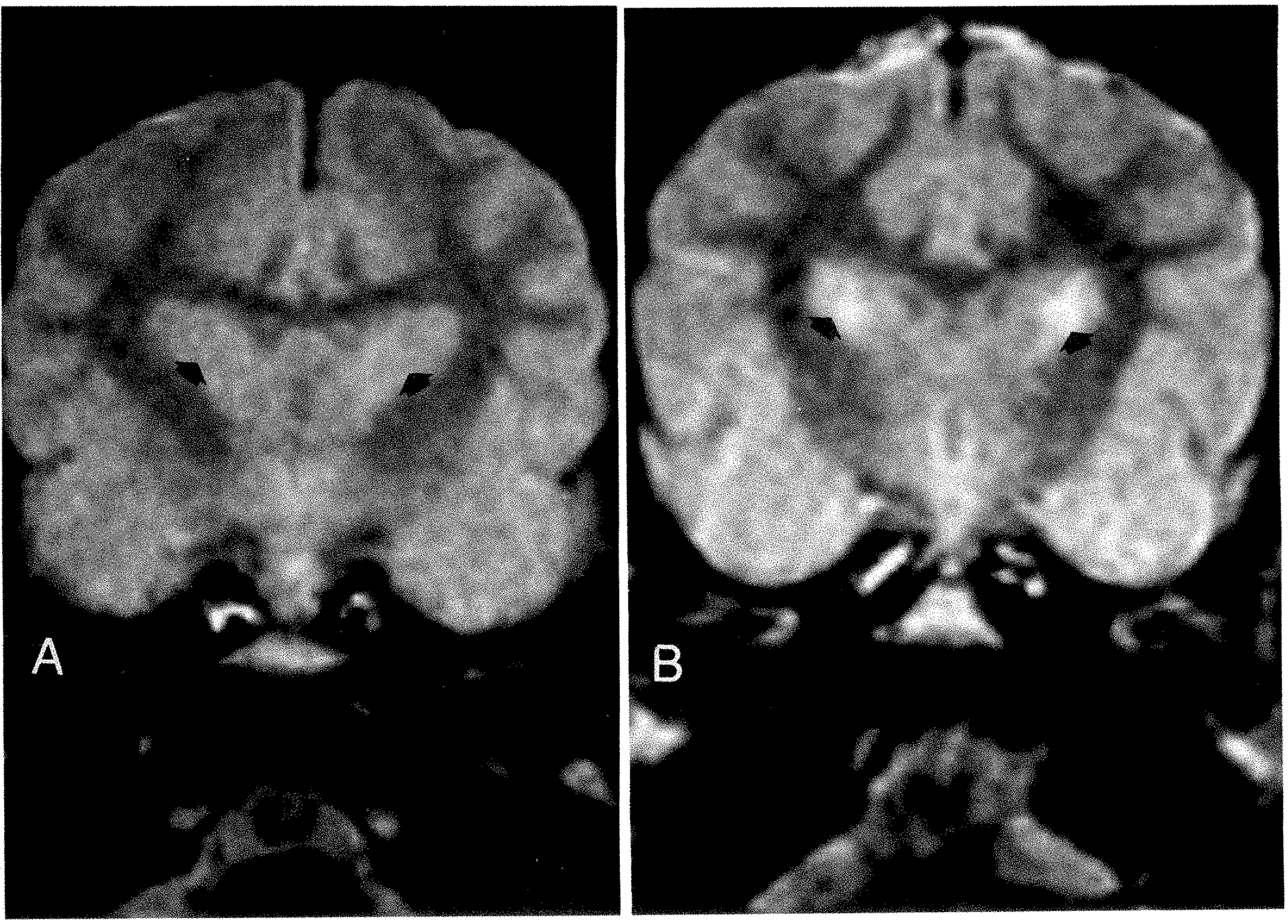

FIG. 5. Representative MRI from two dogs at 7 days recovery from cardiac arrest. Coronal T-2 weighted images are through the level of the head of the caudate nucleus (*arrows*). (A) Normal caudate signal (*arrows*) is seen in this dog after PF. (B) Abnormal hyperintense signal (*arrows*) is visible in bilateral caudates consistent with ischemic injury.

NPF.[12-14,24-26] Unfortunately a number of drawbacks exist in earlier studies: many were carried out more than 10 years ago with now-obsolete equipment; pulsatile flow did not accurately reproduce the normal physiologic state; and conclusions were extrapolated from acutely measured parameters (*i.e.*, early return of electroencephalographic [EEG] activity, blood flow, metabolite levels, etc.) that do not necessarily correlate with eventual neurologic outcome. Most importantly none of these studies examined neurologic function or cerebral pathology after resuscitation from cardiac arrest.

Selection of Experimental Models

This study's objective was to assess neurologic outcome after successful resuscitation from cardiac arrest with either PF or NPF. The study design incorporated a model of global cerebral ischemia with clinical pertinence, objective assessment of well-defined neurologic end points, and circulatory support devices with clinical applicability. Cardiac arrest provided a clinically relevant model of cerebral ischemia.[3,5] One-week survival allowed better interpretations of both functional return and pathologic injury (maturation).[1-3,27] Methods for generating PF and NPF were chosen based on prior validation studies and clinical relevance.

Nonpulsatile flow is a nonphysiologic phenomenon that occurs with application of cardiopulmonary bypass. Furthermore clinical devices used for resuscitative circulatory support are simply modified CPB circuits.[8-11] These factors indicate that CPB should be considered the "standard" for NPF studies. In contrast PF is physiologic and not well reproduced by mechanical blood pumps. Mechanical blood pumps may create pulsatile flow, but its characteristics are often nonphysiologic. The same is not true for mechanical actuation of the heart. Direct mechanical ventricular actuation (DMVA) has been evaluated for more than 25 years and has proven effective in

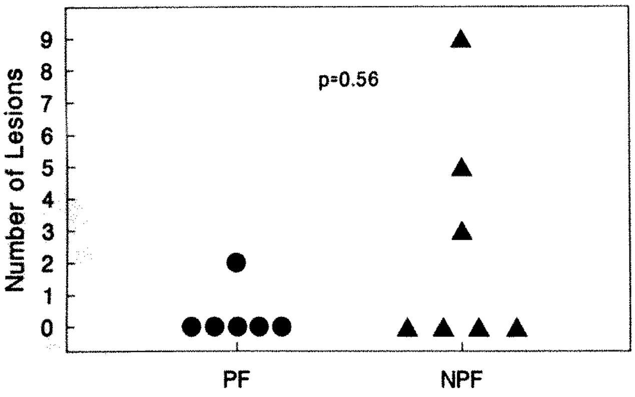

FIG. 6. Brain lesions detected by MRI 7 days after resuscitation with either PF or NPF. Each symbol indicates an individual dog. (PF *versus* NPF, categorical analysis).

providing both acute and prolonged total circulatory support.[15–20] Moreover DMVA reliably generates pulsatile flow, which closely reflects the normal physiologic state. The method has proved to be feasible for resuscitative circulatory support in both laboratory and clinical applications. Therefore DMVA was an acceptable method to generate PF in this comparative study.

Earlier studies compared the hemodynamics and organ perfusion produced by CPB and DMVA. Total circulatory support for up to 4 hours of VF demonstrated comparable hemodynamics and tissue perfusion.[19] Significant differences were found, however, with respect to the myocardium's subsequent tolerance to ischemia. Hearts supported by DMVA had a significantly better tolerance to ischemia than those receiving CPB.[18,20] These effects have been hypothesized to result from differences in PF *versus* NPF. Another underlying factor distinguishing CPB from DMVA is blood contact. Consequences of blood contact in this study would be expected to be relatively minor because circulatory support was implemented for only 1 hour. Both methods facilitate similar flow rates and tissue perfusion without using pharmacologic agents, but their flow dynamics are different. Thus DMVA and CPB are ideal models for contrasting pulsatile and nonpulsatile flow. Differences in neurologic outcome after these two interventions may reflect fundamental distinctions in blood flow dynamics that may be important in the clinical setting.

Neurologic Outcome

Pulsatile flow improved cerebral resuscitation compared with NPF as indicated by consistent differences in each neurologic assessment. Pulsatile flow improved both the recovery rate and the 7-day measure of neurologic function. These findings were paralleled by lower neurologic deficit scores, following PF *versus* NPF. Because each neurologic deficit score implied a grossly obvious abnormality, the implications of these discrepancies may be even greater than suggested by the relative numerical scores. Although other investigators subdivide these scoring systems for better "objectivity" and differentiation,[21–23] this study purposely used more gross interpretations, to avoid ambiguity in assigning scores.

The MRI findings also suggested that PF results in less brain injury, although the between-group differences failed to reach statistical significance in this small group of animals. The detected lesions represent regional edema in areas of neuronal and glial injury.[28] Magnetic resonance imaging is sensitive to such localized abnormalities and provides a useful guide for planning histologic examinations of the brain.

Microscopic evaluations provided the most convincing evidence for the benefits of PF resuscitation. The hippocampus was assessed because of the high vulnerability of the CA1 pyramidal neurons to ischemic injury.[1–3,27–30] Therefore this region serves as an excellent marker for evaluating ischemic brain damage. An important prerequisite for grading CA1 neuronal loss after ischemia is an adequate "maturation" period, because CA1 pyramidal cells take 3 to 4 days to exhibit cell death.[1,3,27] The 8-day survival period in this study allowed ample time for such changes to develop.

CA1 pyramidal neuronal damage was detected in 10 of 15 brains. Both the frequency and degree of injury were significantly greater after NPF. When ischemic injury occurred, it generally manifested in an all or none fashion. These findings suggest either a "fine line" between reversible and irreversible CA1 damage or a profound difference between the two treatment modalities on hippocampal salvage, or perhaps both. The relevance of preserving the hippocampus relates to its functional role in memory. Interestingly the marked sensitivity of this region to ischemia may explain clinical cases of amnesia after resuscitation from cardiac arrest.[29,30]

Caudate sections were assessed because this region is known to be sensitive to ischemic damage[1–3] and because it had relatively frequent MRI abnormalities in the present study. The incidence of histologic lesions was significantly greater after NPF than after PF. Watershed areas of the cerebral cortex showed a similar trend, with lesions found only in dogs experiencing NPF. Lesions in the cortex, however, were markedly less frequent, and between-group differences were not statistically significant for this brain region.

Potential Confounding Factors

The present results indicate that PF improves cerebral resuscitation compared with NPF. Any discrepancies in either prearrest or postarrest conditions need careful assessment to determine whether other variables contributed

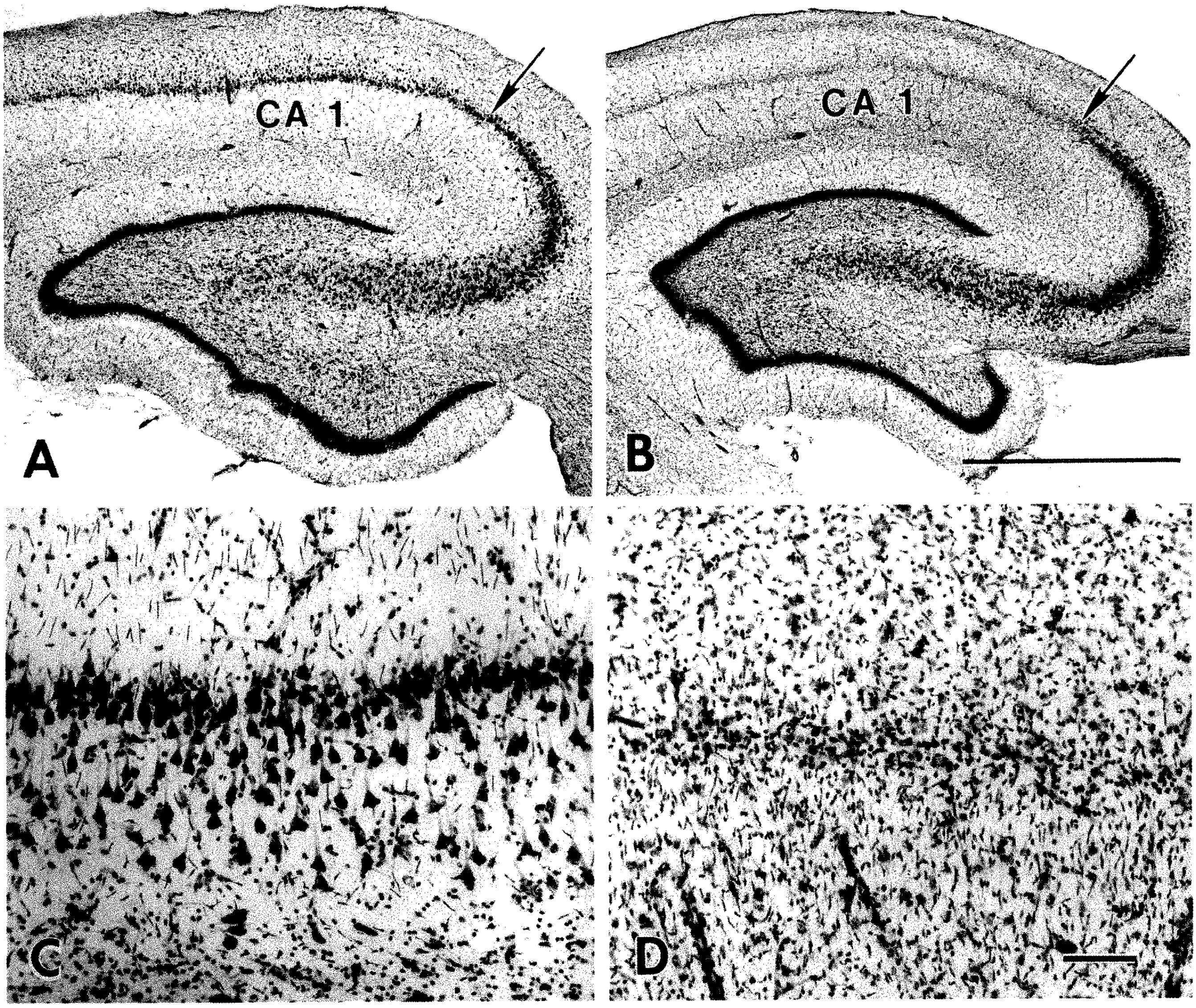

FIG. 7. Photomicrographs of representative hippocampal sections fixed 8 days after resuscitation from cardiac arrest. (A, C) A normal hippocampus (grade 1) from a dog receiving PF. (A) The border of CA1 (*arrow*). (C) At higher power, pyramidal neurons are numerous in CA1. (B, D) A severely damaged hippocampus (grade 5) from a dog receiving NPF. (B) The border of CA1 (*arrow*). (D) At higher power, pyramidal neurons are absent and reactive astrocytes are prominent. Cresyl violet stain. Scale bars = 1.0 mm (A, B) and 0.10 mm (C, D). (D) Pyramidal neurons (grade 5) after NPF.

to this outcome. Care was taken to keep both groups as similar as possible. All animals had normal blood pressures with no hypotensive events. Blood gas parameters were in the physiologic range, with only minor variations between groups. Only arterial blood pressures and core temperatures consistently and significantly differed between the two experimental groups. Differences in arterial pressures were inherent to differences in flow dynamics, whereas temperature differences must be considered a potentially confounding factor.

Both groups were mildly hypothermic before arrest as a result of open thoracotomy. Small differences in prearrest temperatures might have favored the NPF group, because small differences in prearrest temperatures clearly benefit neurologic outcome,[1,3,31,32] but these differences were not statistically significant. The significance of the relatively mild hypothermia (0.5 to 1.1 C) during 15 to 60 minutes reperfusion of the PF animals is uncertain. Immediate cooling by 3 to 4 C during reperfusion has been beneficial in individual[25] but not in all[31] experimental models.

Another factor that deserves consideration is the relative hemodilution that existed during NPF due to the necessary crystalloid prime in the CPB circuit. This condition would be expected to favor NPF, however, because hemodilution has been shown beneficial on cerebral reperfusion and outcome in experimental models using NPF.[33,34] The force required to return blood flow after stasis (yield stress) is

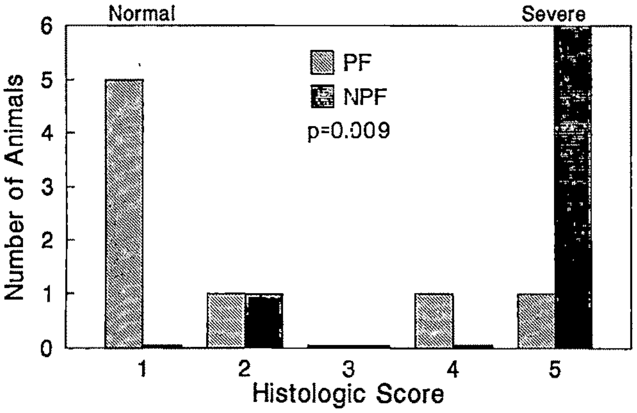

FIG. 8. Bar graph showing the distribution of CA1 histologic scores of the hippocampus in dogs after either PF or NPF for resuscitation. Only PF resulted in normal-appearing CA1 regions (grade 1). Six of seven hippocampi showed complete loss of pyramidal neurons (grade 5) after NPF (PF *versus* NPF, categorical analysis).

increased in proportion to the third power of hematocrit.[35] Furthermore decreased hematocrit is known to lower viscosity, which improves perfusion during NPF.[36] Therefore it is not surprising that studies demonstrating benefits of hemodilution during cerebral reperfusion used NPF. Relative hemodilution after circulatory support was avoided by giving equivalent fluid volumes to both groups. Furthermore 1 unit fresh whole blood was administered after NPF to compensate for potential blood hemolysis.

Hemodynamic Responses to PF Versus *NPF*

Systemic pressures were markedly different between groups during resuscitation. As expected pulse pressures were significantly greater throughout PF reperfusion. Resulting mean arterial pressures were significantly elevated during the first 15 minutes of PF *versus* NPF reperfusion. These pressure differences did not appear to result from discrepancies in total blood flow because: (1) flows were continously adjusted to the greatest degree permissible by venous return; (2) calculated flows were not significantly different; and (3) despite apparent decreases in calculated NPF flows, we found that a calibrated roller pump somewhat underestimates flow, particularly during periods of relative hypotension.

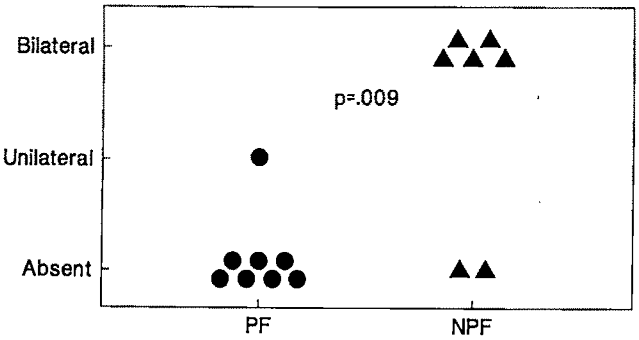

FIG. 9. Ischemic injury in the caudate nucleus after resuscitation with either PF or NPF was determined by examining representative sections. Injury (microinfarcts and/or diffuse neuronal loss) was grouped into categories based on ischemic changes in one (unilateral) or two (bilateral) hemispheres (PF *versus* NPF, categorical analysis; each symbol represents an individual dog).

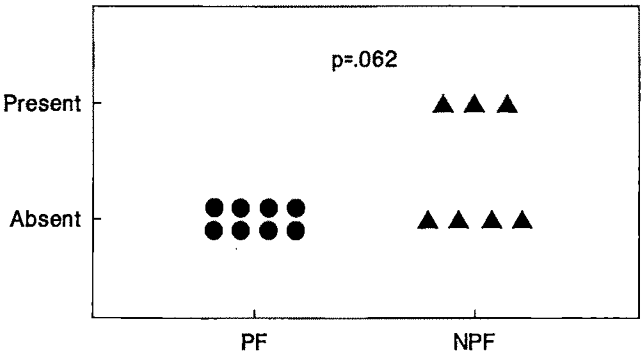

FIG. 10. Both hemispheres of the frontal and parietal cerebral cortices were assessed for ischemic injury after resuscitation using PF or NPF. Only NPF dogs had ischemic findings in one or more representative section (PF *versus* NPF, categorical analysis; each symbol represents an individual dog).

Despite similar flow rates, PF resulted in increased arterial pressures during early resuscitation. Early increases in reperfusion pressures have improved cerebral perfusion and outcome in numerous investigations.[21,31,33,34,37–39] Therefore increased arterial pressures may have been the primary reason for improved neurologic outcome after PF *versus* NPF. Clearly PF was responsible for the early rise in systemic pressures.

Mechanisms responsible for the early rise in systemic blood pressures during PF are not evident from these experimental results. One possible explanation is in the greater energy expenditure required to generate PF, compared with NPF, when mean arterial pressures are kept constant.[40] Extra energy during PF is dissipated in the vascular bed after each pulsation. In resuscitation from cardiac arrest, this component of PF energy may contribute to the return of vascular tone and thereby facilitate an increase in mean arterial pressure.

Pulsatile energy has not been studied after circulatory arrest, but its importance during normal conditions has been strongly emphasized.[41] Dissipation of pulse pressures across capillary beds may contribute to maintainance of capillary bed patency. Likewise PF may aid in reopening capillary beds during reperfusion. Nonpulsatile flow may result in defective capillary perfusion, which may limit recovery of ischemic tissue. The importance of these theoretical effects on cerebral resuscitation is uncertain.

Conclusion

There is a critical time point during global cerebral ischemia after which reperfusion can no longer reverse brain injury. The limits of this poorly understood time frame are in part restricted by the effectiveness of reperfusion. This study demonstrates that cerebral resuscitation is improved by reperfusion with PF compared with NPF and suggests that this effect is probably due to distinctive effects on blood pressure. The simple fact that PF resulted in better neurologic outcome raises questions about the use of NPF for resuscitation after cardiac arrest. In view of these observations, investigators attempting to improve resuscitation from cardiac arrest by use of mechanical circulatory support need to further consider the importance of physiologic pulsatile flow.

References

1. Brierley JB, Graham DI. Hypoxia and vascular disorders of the central nervous system. *In* Adams JH, Corsellis JAN, Duchen LW, eds. Greenfield's Neuropathology, 4th ed. London: Edward Arnold, 1984, pp 125–207.
2. Crain BJ, Westerkam WD, Harrison AH, Nadler JV. Selective neuronal death after transient forebrain ischemia in the mongolian gerbil: a silver impregnation study. Neuroscience 1988; 27:387–402.
3. Bengtsson F, Siesjo BK. Cell damage in cerebral ischemia: physiological, biochemical, and structural aspects. *In* Schurr A, Rigor BM, eds. Cerebral Ischemia and Resuscitation. Boca Raton, FL: CRC Press, 1990, pp 215–233.
4. Lassen NA, Astrup J. Cerebral blood flow: normal regulation and ischemic thresholds. *In* Weinstein PR, Faden AI, eds. Protection of Brain from Ischemia. Baltimore: Williams & Wilkins, 1990, pp 7–19.
5. Safar P. Resuscitation from clinical death: pathophysiologic limits, and therapeutic potentials. Crit Care Med 1988; 16:923–941.
6. Jennings RB, Reimer KA, Steenbergen C. Complete global myocardial ischemia in dogs. Crit Care Med 1988; 16:988–996.
7. Pennington DG, Bernhard WF, Golding LR, Berger RL, Khuri SF, Watson JT. Long-term follow-up of postcardiotomy patients with profound cardiogenic shock treated with ventricular assist devices. Circulation 1985; 72 (suppl 2):II-216–226.
8. Tisherman SA, Grenvik A, Safar P. Cardiopulmonary-cerebral resuscitation: advanced and prolonged life support with emergency cardiopulmonary bypass. Acta Anaesthesiol Scand Suppl 1990; 94:63–72.
9. Reichman RT, Joyo CI, Dembitsky WP, Griffith LD, Adamson RM, Daily PO, Overlie PA, Smith SC Jr, Jaski BE. Improved patient survival after cardiac arrest using a cardiopulmonary support system. Ann Thorac Surg 1990; 49:101–105.
10. Phillips SJ, Zeff RH, Kongtahworn C, Skinner JR, Toon RS, Grignon A, Kennerly RM, Wickemeyer W, Iannone LA. Percutaneous cardiopulmonary bypass: application and indication for use. Ann Thorac Surg 1989; 47:121–123.
11. Mooney MR, Arom KV, Joyce LD, Mooney JF, Golderberg IF, Von Rueden TJ, Emery RW. Emergency cardiopulmonary bypass support in patients with cardiac arrest. J Thorac Cardiovasc Surg 1991; 101:450–454.
12. Hickey PR, Buckley MJ, Philbin DM. Pulsatile and nonpulsatile cardiopulmonary bypass: review of a counterproductive controversy. Ann Thorac Surg 1983; 36:720–737.
13. Mavroudis C. To pulse or not to pulse. Ann Thorac Surg 1978; 25: 259–271.
14. Dernevik L, Arvidsson S, William-Olsson G. Cerebral perfusion in dogs during pulsatile and non pulsatile extracorporeal circulation. J Cardiovasc Surg 1985; 26:32–64.
15. Anstadt MP, Anstadt GL, Lowe JE. Direct mechanical ventricular actuation: a review. Resuscitation 1991; 21:7–23.
16. Lowe JE, Anstadt MP, Van Trigt P, Smith PK, Hendry PJ, Plunkett MD, Anstadt GL. First successful bridge to cardiac transplantation using direct mechanical ventricular actuation. Ann Thorac Surg 1991 (in press).
17. Anstadt MP, Bartlett RL, Malone JP, Brown GR, Martin S, Nolan DJ, Oberheu KH, Anstadt GL. Direct mechanical ventricular actuation for cardiac arrest in humans: a clinical feasibility trial. Chest 1991; 100:86–92.
18. Anstadt MP, Hendry PJ, Plunkett MD, Menius JA Jr, Pacifico AD Jr, Lowe JE. Mechanical myocardial actuation during ventricular fibrillation improves tolerance to ischemia compared with cardiopulmonary bypass. Circulation 1990; 82(Suppl IV): IV-284–290.
19. Anstadt MP, Hendry PJ, Plunkett MD, Menius JA Jr, Pacifico AD Jr, Lowe JE. Mechanical cardiac actuation achieves hemodynamics similar to cardiopulmonary bypass. Surgery 1990; 108: 442–451.
20. Anstadt MP, Hendry PJ, Plunkett MD, Menius JA Jr, Taber J, Tedder M, Lowe JE. Biventricular cardiac assist vs. cardiopulmonary bypas (CPB) for resuscitation. ASAIO Transactions 1991; 37:M518–M519.
21. Sterz F, Leonov Y, Safar P, Radovsky A, Tisherman SA, Oku K. Hypertension with or without hemodilution after cardiac arrest in dogs. Stroke 1990; 21:1178–1184.
22. Leonov Y, Sterz F, Safar P, Radovsky A, Oku K, Tisherman S, Stezoski SW. Mild cerebral hypothermia during and after cardiac arrest improves neurologic outcome in dogs. J Cereb Blood Flow Metab 1990; 10:57–70.
23. Safar P, Abramson NS, Angelos M, Cantadore R, Leonov Y, Levine R, Pretto E, Reich H, Sterz F, Stezoski SW, Tisherman S. Emergency cardiopulmonary bypass for resuscitation from prolonged cardiac arrest. Am J Emerg Med 1990; 8:55–67.
24. Tranmer BI, Gross CE, Kindt GW, Adey GR. Pulsatile versus nonpulsatile blood flow in the treatment of acute cerebral ischemia. Neurosurgery 1986; 19:724–731.
25. Watanabe T, Miura M, Orita H, Kobayasi M, Washio M. Brain tissue pH, oxygen tension, and carbon dioxide tension in profoundly hypothermic cardiopulmonary bypass. J Thorac Cardiovasc Surg 1990; 100:274–280.
26. Andersen K, Waaben J, Husum B, Voldby B, Bodker A, Hansen AJ, Gjedde A. Nonpulsatile cardiopulmonary bypass disrupts the flow-metabolism couple in the brain. J Thorac Cardiovasc Surg 1985; 90:570–579.
27. Van Reempts J, Borgers M. Structural damage in experimental cerebral ischemia. *In* Schurr A, Rigor B, eds. Cerebral Ischemia and Resuscitation. Boca Raton, FL: CRC Press, 1990, pp 235–252.
28. DeLaPaz RL, Shibata D, Steinberg GK, Zarnegar R, George C. Acute cerebral ischemia in rabbits: correlation between MR and histopathology. AJNR 1991; 12:89–95.
29. Volpe BT, Petito CK. Dementia with bilateral medial temporal lobe ischemia. Neurology 1985; 35:1793–1797.
30. Cummings JL, Tomiyasu U, Read S, Benson DF. Amnesia with hippocampal lesions after cardiopulmonary arrest. Neurology 1984; 34:679–681.
31. Martz D, Hoff JT. Physiological protection against cerebral ischemia. *In* Schurr A, Rigor BM, eds. Cerebral Ischemia and Resuscitation. Boca Raton, FL: CRC Press, 1990, pp 337–345.
32. Morawetz RB. Circulatory thresholds of brain dysfunction and infarction. *In* Weinstein PR, Faden AI, eds. Protection of the Brain from Ischemia. Baltimore: Williams & Wilkins, 1990, pp 1–6.
33. Fischer EG, Ames A III. Studies on mechanisms of impairment of cerebral circulation following ischemia: effect of hemodilution and perfusion pressure. Stroke 1972; 3:538–542.
34. Little JR, Rosenfeld JV, Barnett GH. Hemodynamic manipulation in the treatment of brain ischemia. *In* Weinstein PR, Faden AI, eds. Protection of the Brain from Ischemia. Baltimore: Williams & Wilkins, 1990, pp 199–212.
35. Merrill EW. Rheology of blood. Physiol Rev 1969; 49:863–888.
36. Thomas DJ. Whole blood viscosity and cerebral blood flow. Stroke 1982; 13:285–287.
37. Walta AG. Effect of blood pressure on blood flow in ischemic and in nonischemic cerebral cortex. Neurology 1968; 18:613–621.
38. Hossmann V, Hossmann KA. Return of neuronal functions after prolonged cardiac arrest. Brain Res 1973; 60:423–438.
39. Hossmann KA. Hemodynamics of postischemic reperfusion of the brain. *In* Weinstein PR, Faden AI, eds. Protection of the Brain from Ischemia. Baltimore: Williams & Wilkins, 1990, pp 21–36.
40. Shepard RC, Simpson DC, Sharp JF. Energy equivalent pressure. Arch Surg 1966; 93:730–740.
41. Milnor WR. Pulsatile blood flow. Physiol Med 1972; 287:27–34.

DISCUSSION

DR. T. GARDNER (Baltimore, Maryland): Assessment of neurologic function after cerebral ischemia in an experimental animal is a challenging problem. Not only are chronic survival studies after neurologic injury difficult, but the accurate assessment of neurologic function in animals can be very problematic. This study addresses both of these challenges well, and in addition, the investigators chose to assess structural changes in the hippocampal region of the brain, which may be the most sensitive area of the brain to ischemic damage.

This study raises important issues that have been somewhat neglected in cardiac surgery, as we have focused primarily on a heart and not sufficiently on alterations and cerebral metabolism and blood flow during cardiopulmonary bypass and with mechanical assist devices. Although it is understood intuitively that ischemia of any organ results from inadequate absolute blood flow or from inadequate perfusion pressure, we have in fact been led to believe that cerebrovascular autoregulation is so dominant a protective homeostatic mechanism that one does not need to worry about the adequacy of cerebral perfusion under conditions of cardiopulmonary bypass. This study forces us to reexamine this concept.

Duke Cameron and Vincent Tam in our laboratory have examined changes in cerebral blood flow and metabolism associated with cardiopulmonary bypass and with circulatory arrest. Among their observations is that there is some loss of autoregulation of cerebral blood flow on cardiopulmonary bypass at low perfusion pressures and at normothermia. This being the case, the assumption that autoregulation protects the brain during all conditions of bypass may be erroneous and it may be important to examine the precise parameters of perfusion pressure and absolute blood flow more carefully.

Unfortunately in the present study, cerebral blood flow was not directly measured. Rather assumptions about the amount of cerebral blood flow were based on flow calibrations of the perfusion circuit. The observations made in this study would be strengthened considerably if accompanied by measurements of cerebral blood flow.

I would question also the practicality or feasibility of using an open-chested form of resuscitation to achieve better neurologic recovery. Even if pulsatile perfusion with this open-chested cardiac compression device results in better cerebral blood flow than does nonpulsatile flow achieved with a peripheral perfusion pump, there are a number of other mechanisms and variables that could be used to insure better cerebral blood flow with a simple peripheral system. Perfusion variables such as hematocrit, viscosity, pH, carbon dioxide tension, and even perfusion pressure can be altered with most circulatory assist systems and may prove to be more practical mechanisms for improving cerebral blood flow than to use an open-chested resuscitation technique.

Notwithstanding these criticisms, this is an interesting and provocative study and points us in the important direction of looking more carefully at changes in cerebral blood flow and metabolism associated with different techniques of bypass and circulatory support.

DR. CLARENCE DENNIS (Mendota Heights, Minnesota): Although there is a general consensus that pulsataile flow is more physiologic than nonpulsatile flow, it is a pleasure to learn of so convincing a demonstration as has been presented by Dr. Anstadt and his associates.

Some questions arise:

In the late 1930s, Herman Kabat and I published a series of studies of cerebral damage in mongrel dogs occasioned by complete arrest of the cephalic circulation, verified by ophthalmoscopic monitoring of retinal vessels. The studies were undertaken for other reasons but were basic to later observations when extracorporeal circulation was undertaken by Gibbon's group and by our own at Minnesota. The techniques employed pneumatic tourniquet compression encircling the neck with a metal endotracheal tube, a respirator, and a recent cervical laminectomy to permit occlusion of vessels in the spinal canal as well as external to it, all under light barbital anesthesia.

Four minutes of arrest was the maximum period to not leave permanent damage, manifested by vertigo and loss of balance. Eight minutes of arrest was rarely followed by survival. The principal cytologic cerebral damage was loss of Purkinje cells of the cerebellum. No cardiac alteration was seen, and cerebral reperfusion was effected immediately on release of compression.

The questions are:

1. Does the longer period of cerebral ischemia in your experiments relate to some residual flow during the prepump 12.5 minutes, in other words, was total cessation of cerebral flow confirmed by ophthalmoscopy or otherwise?
2. Because we found that puppies tolerated twice the duration of arrest as adult dogs, what was the age of your dogs?
3. Was a respirator operating during the periods of ventricular fibrillation, which might have resulted in some cerebral flow?
4. Did you note Purkinje cell loss such as we found both in our dogs and in human material from senile persons?

DR. R. MORTON BOLMAN (Minneapolis, Minnesota): This is one of the first demonstrations that pulsatile reperfusion allows any advantage over nonpulsatile perfusion in terms of any measurable parameter. As an individual interested in cardiac replacement, I am intrigued by the direct mechanical ventricular actuating device. I would first like to ask the authors whether they have given any consideration to using this device in humans, potentially as a bridge to cardiac transplantation. Secondly what about the use of pulsatile reperfusion for cardiopulmonary bypass? This is an issue that has been debated for many, many years. Should we be using pulsatile reperfusion for bypass, particularly in higher-risk patients or in children who may have longer bypass periods? Also the data presented today are provocative in terms of the potential applicability of pulsatile perfusion in older individuals, in whom we know the incidence of cerebral injury is higher.

DR. FRANK SPENCER (New York, New York): The most serious hazard from bypass is neurologic injury. The frequency of stroke in different reports ranges from as low as 2% to over 5% but, to my knowledge, no one has reported a zero frequency. A focal neurologic deficit is most likely from embolization of some type, such as atheromatous debris from the aortic arch or carotid artery, air, or intracardiac thrombi.

A diffuse nonlocalizing neurologic injury is far more puzzling and almost always remains unexplained. Prospective studies of frequency of neurologic injury from both Scandinavia and England have found a much higher frequency of subtle neurologic and psychometric changes, a frequency as high as 15% to 20%, although fortunately the majority are transient. An uncommon but familiar tragedy, however, is an elderly patient who undergoes a long period of perfusion who either never regains consciousness or has a significant intellectual impairment afterward. Some cardiologists have commented that they are reluctant to have a patient over 70 operated on because of the high frequency of subtle but significant intellectual deterioration afterward. The cause of this unusual but devastating injury is consistent with a diffuse impairment of cerebral blood flow, although other studies have found no consistent impairment of cerebral autoregulation in older patients. The frequency of such injuries increases both with age and duration of perfusion. These diffuse changes, however, correspond well with the elegant histologic studies shown in this report.

I think it is highly probably that the line of investigation being developed by the authors is a very significant one and may lead to findings that could significantly decrease the frequency of neurologic injury with perfusion.

PRESIDENT J. THOMPSON (Galveston, Texas): I would like to ask Dr. Anstadt, while he is coming to the podium, to reflect on what his thought are on the mechanism of this?

We have been told for years that pulsatile is better than nonpulsatile. You made some preliminary speculation that it may be due to the increase in the early pressures.

Have you done studies directed to that? And if not, what studies might you plan to reveal the mechanism for this really startling difference?

DR. Mark P. ANSTADT (Closing discussion): I would like to thank the members and guests for their very courteous remarks. In general I agree with the comments made; however I would like to respond individually to each discussant.

Dr. Gardner, thank you again. I appreciate your statement concerning our evaluation of the hippocampus. We too believe that this is an important region to assess because of its exquisite sensitivity to ischemic damage.

Dr. Gardner discussed the issue of autoregulation, and questioned the protective effects of autoregulatory mechanisms *per se* during either cardiopulmonary bypass or postischemic reperfusion.

I would suggest that autoregulation does not seem satisfactory in the setting of postischemic reperfusion. During early reperfusion autoregulatory mechanisms are literally paralyzed and maximal vasodilatation occurs in the cerebral vasculature. This hyperemic state is followed by what has been termed "delayed postischemic hypoperfusion," where autoregulation returns, but in an aberrant manner. During this period cerebral perfusion is below normal for any given blood pressure and does not exhibit CO_2 reactivity.

Concerning our assessment of cerebral perfusion, slide 42 illustrates cerebral perfusion determined in an earlier study comparing DMVA with cardiopulmonary bypass. As you can see, compared with normal sinus rhythm, there were no significant changes in cerebral perfusion during 4 hours of total circulatory support with either method. Cerebral perfusion tended to decline after 2 hours in the cardiopulmonary bypass group, however, which may have resulted from persistent nonpulsatile flow.

Furthermore previous studies performed in our laboratory and at Ohio State suggest that regional cerebral perfusion is similar during early postarrest reperfusion using either DMVA or cardiopulmonary bypass, despite differences in blood pressure. Therefore factors beyond arterial perfusion may be responsible for the differences we found in neurologic outcome when comparing these two methods.

I appreciate Dr. Dennis's comments concerning his experimentation with a neck tourniquet model to create cerebral ischemia. As in his studies, we also turned the respirator off during experimental ischemia.

Regarding cerebral injury patterns, we believe that some findings using the neck tourniquet model may relate to thromboembolic events induced by tourniquet trauma. We did not find ischemic cerebellar changes in our model of circulatory arrest.

Dr. Bolman, thank you for your questions. Yes we have used this device successfully to bridge to cardiac transplantation. Dr. Lowe and I had this experience at Duke University in February of 1990. The patient, a 56-year-old white woman, underwent successful cardiac transplantation after 2½ days of uncomplicated total circulatory support using DMVA. She is now alive and well more than 14 months after transplantation.

Dr. Spencer, concerning your question of subtle neurologic changes after bypass surgery, we believe that some of these findings are actually related to hippocampal injury. The hippocampus is exquisitely sensitive to ischemia and is functionally responsible for various aspects of memory. Changes in memory are, of course, difficult to assess; clinical case reports, however, indicate an association between amnesia in patients that survive cardiac arrest and ischemic lesions in the hippocampus. Similarly hippocampal injury may explain memory loss in patients after bypass surgery.

Finally regarding mechanisms that may explain the results of this study, I believe that pulsatile flow may improve perfusion characteristics in the cerebral microcirculation as related to the dissipation of pulse pressures across capillary beds. Venous return and, in the case of other vital organs, lymphatic flow also may be improved during pulsatile *versus* nonpulsatile reperfusion. Of course the brain has no lymphatic system. As Dr. Shepard reported in the late 1960s, the energy required to generate pulsatile flow is about 2.2 times greater than that required for nonpulsatile flow, given the same mean arterial pressures. This difference in energy requirements relates to the phasic nature of pulsatile flow. During reperfusion the extra energy required to generate pulsatile blood flow may be used to regain vascular tone and to optimize microvascular perfusion instead of being merely dissipated across capillary beds.

Improved Long-term Survival After Lymphadenectomy of Melanoma Metastatic to Regional Nodes

Analysis of Prognostic Factors in 1134 Patients from the John Wayne Cancer Clinic

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ROBERT M. ELASHOFF, PH.D.,‡ and JAN H. WONG, M.D.*

A review of 1134 patients from the John Wayne Cancer Clinic with melanoma metastatic to regional lymph nodes was carried out to evaluate the importance of various prognostic features after lymphadenectomy. Univariate analysis identified the prognostic significance of clinical stage for lesions with a depth of 0.76 to 4.0 mm ($p = 0.0018$); number of involved nodes ($p = 0.0001$); Breslow's thickness ($p = 0.0487$); gender ($p = 0.0103$); location on an extremity ($p = 0.0104$); synchronous *versus* asynchronous detection of nodal metastases ($p = 0.0107$); age as a continuous variable ($p = 0.0670$); and unknown primary site ($p = 0.088$). Multifactorial analysis showed that number of involved nodes ($p = 0.0001$), extremity location of primary ($p = 0.0059$), and Breslow thickness ($p = 0.0334$) maintained their significance, whereas gender ($p = 0.0627$) and clinical stage ($p = 0.0942$) were almost significant. The long-term survival of the entire patient population at 5, 10, and 15 years of follow-up was estimated to be 46%, 41%, and 38%. When individual characteristics found to be significant by multivariate analysis were combined into different subsets, there was considerable heterogeneity, with 5-year survival varying from 79% to 14%. To quantify this heterogeneity better, a mathematical model was developed and found to approximate closely the observed survival rates in the heterogenous subsets and in the group as a whole.

THE INCIDENCE OF cutaneous melanoma is increasing throughout the world, particularly in those locations where people with fair complexions are exposed to sunlight. This annual rate of increase, 4%, is higher than for any other malignancy. It has been estimated that by the year 2000 one in every 90 individuals in the population will develop melanoma.[1] The presence of lymph node metastases is well established as a crucial prognostic indicator in this disease. Long-term survival after lymphadenectomy of patients with lymph node metastases (American Joint Committee on Staging [AJC] stage III) is poor, with less than 15% of patients surviving 10 years or longer in most series.[1,2] The number of involved nodes, the Breslow thickness,[3] anatomic location of the primary site, clinical status of nodes, and gender are among the factors most frequently reported to be of prognostic significance in AJC stage III disease.[1,2,4,5] The observation that survival of patients with lymph node metastases treated at the John Wayne Cancer Clinic (JWCC) appears to be higher than previously reported from other series,[5] including our own early experience, led us to review our entire experience with this stage of the disease in an attempt to explain the apparent improvement in survival rates. We undertook a univariate and multivariate analysis of the prognostic factors significant in this disease and developed a prognostic model that was predictive of survival in our patients as a whole group, as well as in the heterogenous subsets.

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Material and Methods

Patient Population

The clinical records of 5111 patients treated at the John Wayne Cancer Clinic during the 18-year period between April 1971 and January 1, 1989 were reviewed and registered into the JWCC's computerized database developed at the John Wayne Cancer Clinic. The period of follow-up observation ranged from 2 to 20 years, with only 47 patients (0.92%) in the entire series being lost to follow-up.

Regional lymph node metastases were found in 1134 patients; and 584 patients (51.1%) died. Among these

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complete information was available on 737 patients regarding age, gender, anatomic location, Clark's level, and Breslow thickness of the primary site, number of involved nodes, clinical stage (I or II), timing of lymphadenectomy for clinical stage II disease, whether synchronous or asynchronous, and whether or not the patient received adjuvant immunotherapy following lymphadenectomy. This group of 737 patients was used to evaluate the significance of the various prognostic factors by univariate and multivariate analysis.

Pathology

Examination of the lymph nodes was performed using routine techniques of hematoxylin and eosin staining. In approximately 90% of patients, the number of lymph nodes containing metastatic melanoma could be accurately determined. The primary lesions were examined by the pathology staff of the Department of Pathology at UCLA, and their interpretation, as presented on the pathology report, was used as the basis of this analysis. Unfortunately data were often not uniformly recorded on certain histologic variables, such as the degree of ulceration, lymphocytic infiltration, mitotic index, and Breslow thickness of the primary lesion. From 1971 to 1978, Dr. Wallace Clark was consulted regarding the interpretation of the Breslow thickness or Clark's level of the primary when questions were raised by the staff pathologist.[6] During the past 10 years, these questions were reviewed by Dr. Alistair J. Cochran, who is currently undertaking a detailed prospective review of the histologic variables of the primary melanoma, which will be the subject of a subsequent report.

Treatment

Patients with primary lesions underwent wide excision of the primary site, according to the guidelines we have previously described.[7,8] All patients with lymph node metastases were treated with regional lymphadenectomy. Our standard operative approach for radical lymphadenectomy at various sites has been previously described.[7,9,10] Because they are at high risk for the future development of metastatic disease, many of these patients participated in adjuvant immunotherapy protocols. Approximately 55% of patients received adjuvant immunotherapy consisting of either nonspecific immunotherapy with bacille Calmette-Guérin administered by the tine technique, as previously described,[11] or active specific immunotherapy with tumor cell vaccine administered by the intradermal or intralymphatic routes.[12] Because our approach to the management of primary melanoma at various anatomic sites has been previously reported,[7] it is only briefly reviewed here with regard to special sites.

Regional Lymph Node Metastases from Melanoma of an Unknown Primary Site

A special group of patients present with regional lymph node metastasis, but without a determined site of the primary melanoma. The incidence of these patients is approximately 14% (155/1134) of all patients with lymph node metastases seen in the JWCC. Although logic suggests that these patients might fare worse than those with a known primary, a review of our experience has found their survival rate to be equal or superior to that of patients with a known primary and, in this analysis, equal to those with thin primary lesions on the extremity ($p = 0.08$). Because prognosis was nearly the same for both groups of patients, we advocate the same aggressive regional lymphadenectomy for patients with unknown primary melanoma as for those with a known primary.[13]

Melanomas Arising from the Axial Location on the Trunk or Head and Neck Primary Sites

Melanomas arising on the axial skin of the trunk, particularly those near the midline or belt line, or those near the neck, may drain to one or more lymphatic areas. Sappey[14] defined a line that is 2 cm wide and encircles the trunk at the level above and below the umbilicus. Lesions above this line generally drain to the axillary nodes, and those below the line drain to the inguinal nodes. Certain of the midline lesions, particularly those of the umbilical area or midline of the back, can drain to all four areas, however. This problem of ambiguous lymphatic drainage is one argument advanced against elective lymph node dissection (ELND) for truncal melanomas. We introduced cutaneous lymphascintigraphy in 1977 to solve this problem.[15-17] Although we initially used colloid gold, we now use a technetium (^{99}Tc)-labeled dextran lymphatic scan, during which ^{99}Tc is injected into the primary melanoma site to determine into which lymph node basins the primary melanoma might drain.[18] The procedure does not indicate nodal metastasis, but does accurately demonstrate the route of lymphatic drainage of the primary site. This technique is used to select which regional lymph nodes to remove by lymphadenectomy.

Statistical Methods

Estimated survival rates were obtained by the nonparametric Kaplan-Meier method.[19] The log-rank test was used to determine differences in survival of patients from subgroups defined by different levels of risk factors. This method of univariate analysis is useful when all variables are categorized into subgroups that are maximally separated in terms of survival rates. For example if the survival rate of patients with truncal lesions is not statistically different from that of patients with head and neck lesions, but is statistically different from that of patients with extremity lesions, the first two groups are combined and compared with the third. This technique was used in part to define categories for the risk factors tumor depth, number of nodes, and tumor location. A more general rank test than the log-rank test was used to test for multivariate association of the risk factors. A discussion of these tests can be found in Kalbfleisch and Prentice.[20] The multivariate results were confirmed using the Cox proportional hazards regression model.[21] The statistical package of SAS procedures LIFETEST[22] and PHGLM[23] were used in the univariate and multivariate analyses. Survival time was defined as the time a patient remained alive after the documented date of nodal metastatic disease at lymphadenectomy.

Results

Long-Term Survival

Table 1 shows long-term survival rates after lymphadenectomy of patients with lymph node metastases and indicates that survival at 5, 10, and 15 years was 46%, 41%, and 38%, respectively. Note that the number of patients still available for observation beyond 10 years was 197, whereas it was 87 for more than 15 years. The rate of deaths per unit of time changes dramatically after 5 years to 1% per year between years 5 and 10, and then to only 0.6% per year thereafter.

Univariate Analysis of Prognostic Factors

Table 2 lists the factors analyzed by univariate analysis. The univariate analysis shows the following factors to be of prognostic value: (1) numbers of involved nodes categorized as 1 *versus* 2 to 4 *versus* 5+ nodes (p = 0.0001); (2) extremity location of primary (p = 0104); (3) thickness of the primary lesion categorized as 0.1 to 1.49 *versus* 1.5 to 3.99 *versus* 4.0 mm (p = 0.0478); (4) gender (p = 0.0130); (5) synchronous rather than asynchronous metastases (p = 0.0107); (6) age as a continuous variable (p = 0.067); (7) clinical stage at the time of lymphadenectomy (I *vs.* II) was of prognostic significance for lesions of Breslow thickness from 0.76 to 4.0 mm (p = 0.0016) (Fig. 1), but not for those greater than 4.0 mm (p = 0.2960); and (8) unknown primary site (p = 0.0876).

TABLE 1. *Long-Term Survival of 1134 Patients with Melanoma Metastases to the Lymph Nodes Treated at the John Wayne Cancer Clinic*

Time (yr)	Survival (%)	SD	No. Dead*	No. Observed†
1	84	1.1	175	902
2	66	1.5	362	647
3	55	1.6	461	496
5	46	1.6	537	357
10	41	1.7	573	197
15	38	1.8	582	87

* Cumulative number of deaths up to the time point.
† Number of patients still under observation beyond the time point.

TABLE 2. *Analysis of Prognostic Factors by Univariate and Multivariate Methods*

	p	
Factor	Univariate	Multivariate*
No. of nodes	0.0001	0.0001
Breslow thickness	0.0487	0.0334
Sex	0.0103	0.0627
Age	0.0670	
Location of primary lesion		
Extremity	0.0104	0.0059
Unknown	0.0876	
Head and neck	0.1845	
Trunk	0.1168	
Clinical stage I *vs.* stage II		
0.76–4.00 mm	0.0016	0.0942
≥4.00 mm	0.2960	
Synchronous *vs.* metachronous	0.0170	
Clarks's level	0.3685	
Adjuvant immunotherapy	0.4118	

* Data shown only for factors with $p < 0.10$ by multivariate analysis.

Prognostic Factors Significant by Multivariate Analysis

The eight factors found to be most significant by univariate analysis were examined by multivariate analysis because there could be other interrelationships between these variables. For example there is a well-known relationship between the anatomic location of the primary site and gender; locations on the extremity are more frequent in women than in men. Therefore a multifactorial analysis was performed to examine primary predictive factors that independently correlated with survival rates, simultaneously accounting for the contribution made by the factors, which was found to be significant by univariate analysis. Each variable was analyzed in sequence for its

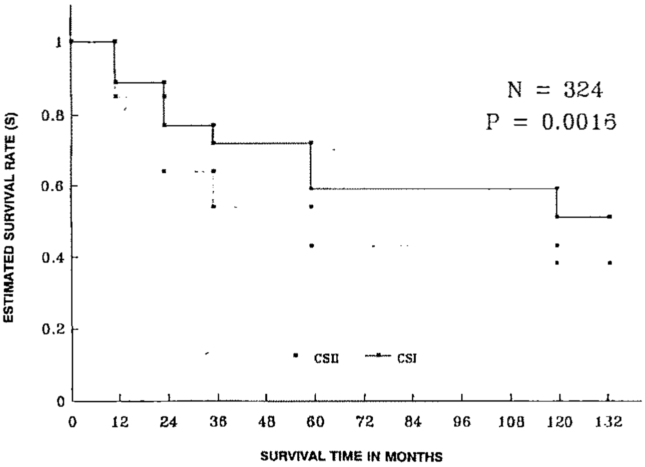


FIG. 1. Long-term survival rates of patients with lymph node metastases undergoing lymphadenectomy for clinical Stage I *versus* II disease (primary melanoma depth 0.76 to 4.0 mm).

additive prognostic value after the previously added factors had been taken into account. A general multivariate rank test was used[20] as well as the Cox proportional hazards regression model. Both methods use a forward variable selection procedure, and they provided the same results. This multifactorial analysis, which is summarized in Table 2, showed that only the number of involved nodes ($p = 0.001$), the location on an extremity ($p = 0.0059$), the depth of the primary ($p = 0.0334$), the patient's sex ($p = 0.0627$), and clinical stage ($p = 0.0942$) were dominant variables. All other factors had a p value greater than 0.10.

Development of Mathematical Prognostic Model

We next attempted to determine the importance of each of these variables in overall survival for the group. In so doing it became evident that there were some subsets of patients with regional nodal metastases who had different degrees of risk of dying from their disease. Figure 2 gives a summary of the 5-year survival rates categorized by gender, primary site, number of nodes, and thickness. Taking the standard errors into account, the 5-year rates for nonextremity lesions do not vary significantly for men and women, whereas Breslow thickness and the number of involved nodes are highly significant prognostic factors for these sites. We concluded that gender was not a prognostic factor for primary melanoma sites of the head and neck or trunk, and male and female subgroups for these sites could be combined as shown in Figure 3. For extremity melanomas and those with unknown primary site, however, the significant prognostic factors vary with gender; the number of involved nodes are important factors in women, but Breslow thickness is more significant in men. Male patients with an unknown primary site were similar to those with thin-extremity melanomas. These various prognostic factors can be combined to create three categories of melanoma patients with lymph node metastases: low risk, with expected survival of 65–75%; middle risk, with expected survival of 40% to 55%; and high risk, with expected survival of 25% to 30%.

Next we developed a mathematical model based on the prognostic value that could be placed on each of these variables and their interactions. The model is defined as a mixed exponential model. In a regular exponential model, the survival rate is defined as

$$S(t) = \exp(-\alpha t),\ t > 0,$$

where α is the hazard rate that is assumed to be constant. In a mixed exponential model, the survival rate is defined as a function of two rates and is a mixture of two different survival functions. A mixing proportion is needed so that the survival rate is always bounded between 0 and 1, in other words, $0 \leq S(t) \leq 1$. The general form of a mixed exponential model is

$$S(t) = p\exp(-\alpha_1 t) + (1-p)\exp(-\alpha_2 t),\ t > 0,$$

where α_1 and α_2 are two different rates and p is the mixing proportion. Risk factors can be included into the model by defining the rates α_1 and α_2 in terms of the risk factors. As illustrated in Figure 2, we saw many complex interactions between the risk factors that affected survival. For example there was no gender effect for patients whose primary lesion was in the extremity, although depth and the number of involved nodes were still independently important. Therefore a term was included in the model that accounts for the interaction between the lesion lo-

Extremity and unknown sites

	Males		Females	
	<1.5 mm (N)	≥1.5 mm (N)	<1.5 mm (N)	≥1.5 mm (N)
1 node	50% ± 8 (49)	45% ± 10 (29)	66% ± 7 (53)	79% ± 9 (24)
2–4 nodes	57% ± 7 (52)	48% ± 14 (20)	67% ± 8 (31)	54% ± 11 (25)
5+ nodes	46% ± 9 (32)	14% ± 13 (13)	63% ± 12 (17)	57% ± 15 (16)

Nonextremity site

	Males		Females	
	<1.5 mm (N)	≥1.5 mm (N)	<1.5 mm (N)	≥1.5 mm (N)
1 node	73% ± 8 (45)	49% ± 7 (75)	76% ± 12 (14)	45% ± 13 (23)
2–4 nodes	47% ± 10 (33)	32% ± 6 (77)	49% ± 16 (11)	35% ± 12 (22)
5+ nodes	21% ± 9 (26)	34% ± 9 (32)	44% ± 17 (10)	14% ± 13 (8)

FIG. 2. Five-year survival rate of patients with lymph node metastases according to prognostic factors.

Extremity and unknown sites

Male*

<1.5 mm	≥1.5 mm
50%	40%

Female†

1 node	2–4 nodes	5+ nodes
70%	60%	55%

Nonextremity site‡

	<1.5 mm	≥1.5 mm
1 node	70%	50%
2–4 nodes	50%	30%
5+ nodes	30%	25%

* Number of involved nodes is insignificant for males.

† Thickness is insignificant for females.

‡ Sex is an insignificant factor for nonextremity lesions.

FIG. 3. Five-year survival rate of patients with lymph node metastases according to prognostic factors.

cation and gender. For patients with nonextremity lesions, the relationships between gender, depth, and the number of nodes was more complicated because gender did not appear to be of importance for nonextremity sites. In particular we saw that depth was not a prognostic factor for females, but that the number of nodes remained important. Males with nonextremity lesions were observed to have different rates of survival for different thicknesses but the number of nodes did not decrease the survival rates as expected.

Table 3 gives the coding of all risk factors and interaction factors in the model. Three interaction variables, extremity and gender (X_7), gender and node (X_8), and gender and depth (X_9), account for the complex interactions we observed in this dataset. For example the interaction variable X_7 is 0 when the variable indicating tumor location (X_5) is 0. This means that the site of the lesion is not on an extremity and indicates that the effect of gender is 0. The 5-year survival rates of the JWCC database confirm this observation. In particular the estimated mixed exponential model is defined as:

$$\hat{S}(t) = 0.7 \exp(-\hat{\alpha}_1 t) + 0.3 \exp(-\hat{\alpha}_2 t)\ t > 0,$$

where

$$\ln(\hat{\alpha}_1) = \exp[-(3.864 - [0.511{*}X_3 + 0.7388{*}X_4]{*}X_8 - [0.4173{*}X_2]{*}X_9 + 0.08{*}X_6 + 0.59{*}X_7)]$$

and

$$\ln(\hat{\alpha}_2) = \exp[-(7.327 - [0.417{*}X_3 + 0.6732{*}X_4]{*}X_8 - [0.4321{*}X_2]{*}X_9 + 0.19{*}X_6 + 0.071{*}X_7)].$$

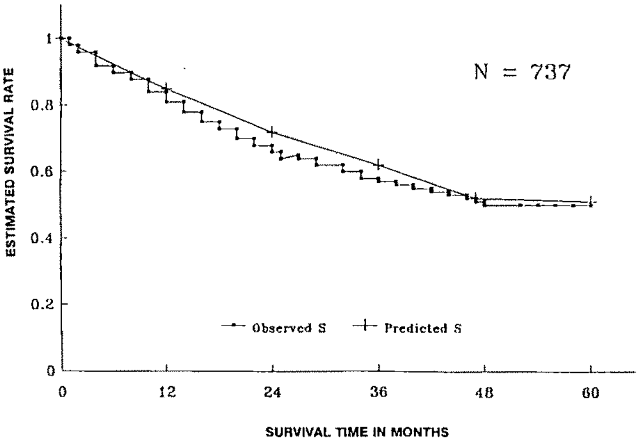
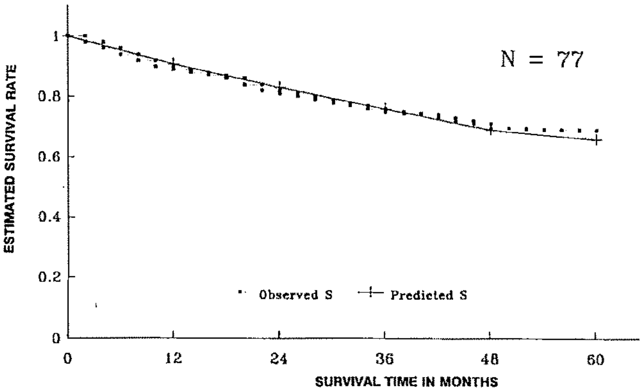
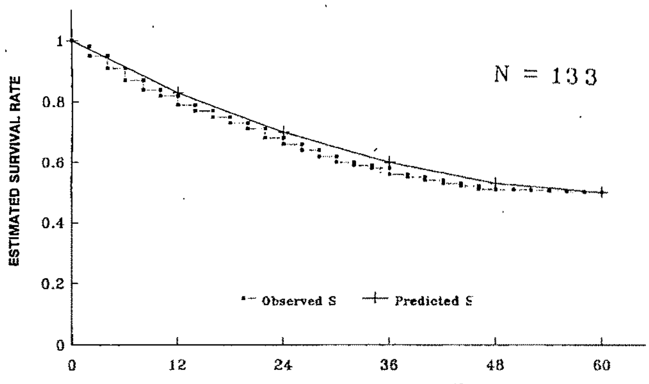


FIG. 4. Observed and predicted survival rates for all patients.



* Low risk status defined by extremity location, female gender, and one involved lymph node

FIG. 5. Observed and predicted survival rates for low-risk group.



*Middle risk defined by extremity location, male gender and thin lesions (<1.5mm)

FIG. 6. Observed and predicted survival rates for middle-risk group.

Comparison of Observed Survival with That Predicted by the Mathematical Model

Figure 4 gives the overall observed survival *versus* that expected by our model. Figures 5, 6, and 7 compare the observed *versus* the expected survivals for selected subsets of patients at low, medium, and high risk. It will be noted that the mathematical model closely parallels the observed survival and does not significantly deviate from it during the first 5 years. After 5 years the observed risk can be accurately estimated by using a risk factor of 1% per year between years 5 and 10.

Discussion

In the present study, the prognosis of patients with metastases to the regional lymph nodes depended on a number of factors, the most important of which are the number of involved lymph nodes, the depth of invasion of the primary melanoma, the sex of the patient, and the site of the primary melanoma. As clearly indicated by this study, there is great heterogeneity in the prognosis of patients with lymph node metastases; some subsets may have expected survival rates of as high as 79%; whereas others may be as low as 14% (Fig. 2). Thus it is critical that these important prognostic factors be considered when giving prognostic information to the individual patient, as well as when evaluating the results of trials of adjuvant therapy.

TABLE 3. *Coding of Risk Factors for the Mathematical Model*

Factor	Defined Factor (X_i) in the Model	Coding for the Factor X_i
Sex	X_1	1 = F; −1 = M
Tumor depth	X_2	0 = <1.5 mm 1 = ≥1.5 mm
No. of involved lymph nodes	X_3	1 = 2–4 nodes 0 = other than 2–4 nodes
	X_4	1 = 5+ nodes 0 = other than 5+ nodes
Lesion location	X_5	1 = extremity 0 = nonextremity
Survival time	X_6	1 = patient still alive after 36 mo 0 = patient not observed after 36 mo
Interaction variables with lesion location		
Sex	X_7	0 = nonextremity −1 = extremity, M 1 = extremity, F
Sex and nodes	X_8	0 = extremity, M 1 = extremity, F
Sex and tumor depth	X_9	0 = extremity, F 1 = extremity, M

The present study confirms our initial impression that the overall survival results have improved to a 5-year survival rate of 46% compared with our earlier series in which only 37% of 150 patients with melanoma who were seen between 1954 and 1976 survived.[5] These patients were treated by lymphadenectomy but received neither chemotherapy nor immunotherapy. The clinical course of these patients up to the point of the development of post-lymphadenectomy metastases was representative of the "natural history" of the disease treated by surgery alone. The explanation for this improvement is not readily evident, because the distribution of patients with regard to nodal status and other features is similar.

This study confirmed the predictive significance of the number of tumor-positive nodes and thickness of the primary melanoma. The number of positive lymph nodes appears to be a measure of tumor burden and their metastatic potential and served to show that the primary melanoma could produce cells capable not only of reaching the lymph nodes but also of proliferating and surviving in them. Alternatively the number of positive nodes may indicate that the patient's capacity to combat the melanoma's ability to spread is relatively limited. More recent studies from our laboratory have indicated the immunodepression exhibited by the lymph nodes draining the primary melanoma.[22]

The greatest difference in survival observed in our early report was between patients with one tumorous node and those who had five or more positive nodes. In univariate analysis characteristics of the thickness of the primary, the absence of ulceration, and the micrometric measurement of ulceration were prognostic for survival. In the multivariate analysis, only thickness was of prognostic significance, because the presence and extent of ulceration were closely related to the measurement of thickness. It is of interest to note that these characteristics of the primary tumor also could predict survival in a manner similar to the time-honored parameter of tumor burden in the nodes. In fact thickness appears to be more important than the number of nodes for extremity lesions in men. It is likely that these characteristics correlated with the aggressiveness and duration of the primary melanoma and with the number of tumor cells present. The larger the number of tumor cells, the greater the chance of phenotypic alterations that permit cells to spread and metastasize. When analyzed separately in the univariate test, other factors including age, gender, whether or not lymph nodes were clinically palpable before lymphadenectomy, location of the primary lesion, presence of satellitosis, type of biopsy (incision/excision), histogenetic pattern, level of invasion, and frequency of mitoses in the primary tumor, did not disclose any statistically significant differences in survival among patients within the different subcategories. By multivariate analysis, however, the importance of the location of the primary after adjusting for primary thickness and the number of tumor-positive nodes yielded a p value that was highly significant. The volume of metastic melanoma in each involved node that had previously been found to be highly significant could not be accurately evaluated in this retrospective study,[24] except indirectly by the number of involved nodes and their clinical status (I *vs.* II).

The large number of patients in this series, which we believe to be the most yet reported, allowed us to evaluate a greater number of variables by multivariate analysis, while maintaining adequate sample sizes to ensure statistical power. We observed remarkable heterogeneity, however, between various subsets of patients based on the prognostic factors found to be significant by multivariate analysis. This can best be observed in Figure 3. The tremendous variation between a 70% survival in the low-risk group *versus* only 25% to 30% survival in the high-risk group indicates the importance of accurately quantifying these factors when comparing series of patients with lymph node metastases. This is especially important when evaluating the results of adjuvant therapy where variations in the natural history of the disease managed by surgery alone are so heterogeneous that it probably exceeds the expected effect of most adjuvant therapies.

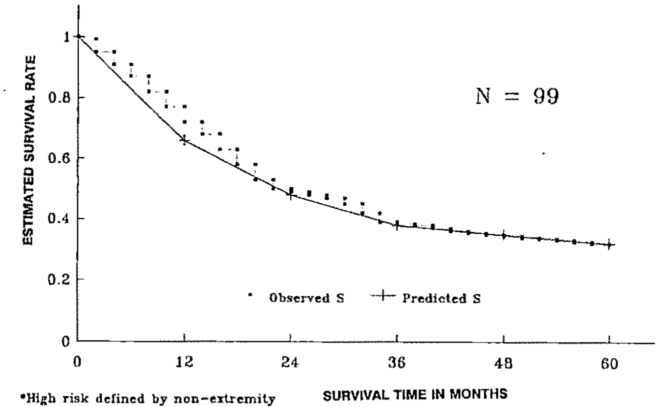
Therapeutic Lymphadenectomy

There is general agreement that regional lymphadenectomy is indicated for the patient who has clinically suspicious or pathologically proved metastasis to regional lymph nodes. Contrary to the pessimism in the early literature, we found that 38% of such patients will survive 10 years, and the patients with thin melanoma and only one clinically positive lymph node may have a 5-year survival as high as 79%. Therefore patients with clinically involved regional nodes should not be considered as being incurable due to occult distant metastatic disease, because a significant proportion can benefit from surgical resection.

Results of Therapeutic Versus *Elective Lymphadenectomy: Clinical Status of Regional Nodes—Clinical Stage I* Versus *II*

Our results indicate a highly significant improved survival for clinical stage I (CS-I) patients with lymph node metastases who received elective lymphadenectomy (ELND) *versus* clinical stage II (CS-II) patients who received therapeutic lymphadenectomy (TLND).[1,4] Five- and ten-year survival for ELND was 59% and 51% *versus* 43% and 38%, respectively, for TLND. This fact forms the basis for the frequent recommendation of ELND in patients with intermediate-depth primary melanomas.[26] On initial examination, however, almost 90% of melanoma patients have localized disease without clinical evidence of metastases to the regional lymph nodes or distant sites. Such CS-I patients present a common therapeutic dilemma regarding the management of the regional nodes. Some oncologists advise immediate ELND for these patients. Others adopt a program of "watch and wait" with careful follow-up examinations and delayed TLND if metastases develop, because such a small proportion of CS-I patients have microscopic metastases present in the regional nodes. Thus the benefit of ELND for patients who are CS-I is controversial. Most large, long-term, retrospective, biostatistical studies from single institutions have demonstrated a small but significant therapeutic benefit from ELND for patients with CS-I melanomas of an intermediate Clark's level or Breslow thickness.[27,28] Furthermore the survival rates after TLND average 15% to 25% higher for patients who are CS-I with clinically occult metastases to the regional lymph nodes (pathological stage II, PS-II) than for patients who are CS-II, a fact we have confirmed in this study (Fig. 1). This suggests that such CS-I and PS-II patients may benefit from early ELND.

In spite of this apparent benefit of ELND, randomized clinical trials have not demonstrated a statistically significant overall survival advantage from ELND for all patients with CS-I melanoma.[29-32] Even the randomized trial by the World Health Organization, however, found that patients with intermediate level melanoma of the extremities who underwent ELND had survival rates approximately 11% higher than patients who underwent delayed TLND.[30-32] This difference was not statistically significant



*High risk defined by non-extremity location, thicker lesions ($\geq$ 1.5mm) and 2–4 involved lymph nodes

FIG. 7. Observed and predicted survival rates for high-risk group.

because there were small numbers of patients in this subset of the trial. Biostatistical considerations require the comparison of large numbers of patients to test the null hypothesis. Two such large, randomized trials are currently under way,[28] but the results will not be available for many years.

Intraoperative Lymphatic Mapping and Selective Lymphadenectomy

We have developed a method to identify, within the total population of CS-I melanoma patients, those who have nodal metastases, because they are the only ones that can benefit from ELND. In 1977 we introduced cutaneous lymphoscintigraphy[15–17] as a methodology for identifying the regional lymph basin of primary drainage for melanomas located in ambiguous sites, such as the midline of the trunk or the shoulder. We hypothesized that new operative techniques developed to identify the lymphatic drainage path from the site of the primary melanoma to the individual lymph nodes within the lymphatic basin might indicate those nodes most likely to contain metastatic melanoma. We have previously described our studies of intraoperative mapping of the regional lymphatics and demonstrate the procedure's high degree of accuracy in identifying those patients who have metastases in the regional lymph nodes.[33,34] Intraoperative mapping permits selective lymphadenectomy of sentinel draining lymph nodes, intraoperative frozen section, and immunohistochemistry, resulting in accurate pathologic staging. Thus surgeons can select those patients who are PS-II for immediate therapeutic lymph node dissection.

Using these techniques we have found that only 20% of CS-I melanoma patients have micrometastases to the regional lymph nodes that are detectable either by routine hematoxylin and eosin staining or by special immunohistochemical techniques. This 20% of patients then can be subjected to standard lymphadenectomy, whereas the other 80% of patients without nodal metastases can avoid the complications of radical lymphadenectomy.

We were surprised by the great heterogeneity in survival rates among patients with lymph node metastases, because our prejudice was that most factors were of little importance except for the tumor burden, as judged by the numbers of involved nodes and their clinical status. Our results indicate, however, that multiple prognostic factors are involved and must be considered in providing prognostic advice to each patient. The mathematical model that we have derived appears to be remarkably accurate in predicting survival of the various subsets of patients in our large data set. The most critical question, however, relates to the general applicability of this model to other data sets. This needs to be accomplished before it can be used for analysis of risk for adjuvant trials or prognosis of individual patients. We are optimistic that the combination of clinical pathologic features as quantitated by our model can be combined with certain serum markers of immunoprognostic importance that we have developed to predict more accurately the natural history of this erratic disease in an individual patient.[35] Unfortunately in a retrospective analysis of this type many features of the primary lesion, such as Balch's ulceration, mitotic index, or lymphocytic infiltration, could not be adequately evaluated. We expect this will be remedied by a detailed prospective evaluation of such factors currently under way by Dr. Alistair J. Cochran.

REFERENCES

1. Balch CM, Soong SJ, Shaw HM, et al. An analysis of prognostic factors in 4000 patients with cutaneous melanoma. *In* Balch CM, Milton GW, eds. Cutaneous Melanoma: Clinical Management and Treatment Results Worldwide. Philadelphia: JB Lippincott, 1985, pp 321–352.
2. Balch CM, Soong SJ, Murad TM, et al. A multifactorial analysis of melanoma: III. Prognostic factors in melanoma patients with lymph node metastases (stage II). Ann Surg 1981; 193(3):377–388.
3. Breslow A. Thickness, cross sectional area, and depth of invasion in the prognosis of cutaneous melanoma. Ann Surg 1970; 172:902–908.
4. Roses DF, Provet JA, Harris MN, et al. Prognosis of patients with pathologic stage II cutaneous malignant melanoma. Ann Surg 1985; 201:103–107.
5. Callery C, Cochran AJ, Roe DJ, et al. Factors prognostic for survival in patients with malignant melanoma spread to the regional lymph nodes. Ann Surg 1982; 196:69–75.
6. Suffin SC, Waisman J, Clark WH Jr, Morton DL. Comparison of the classification by microscopic level (stage) of malignant melanoma by three independent groups of pathologists. Cancer 1977; 40:3112–3114.
7. Morton DL. Surgical treatment for malignant melanoma. Clin Oncol 1984; 3:517–529.
8. Morton DL. Current management of malignant melanoma. Ann Surg 1990; 212(2):123–124.
9. Finck SJ, Giuliano AE, Mann BD, Morton DL. Results of ilioinguinal dissection for stage II melanoma. Ann Surg 1982; 196:180–186.
10. Storm FK, Eilber FR, Morton DL, Clark WH Jr. Malignant melanoma of the head and neck. Head Neck Surg 1978; 1:123–128.
11. Morton DL, Eilber FR, Holmes EC, et al. BCG immunotherapy of malignant melanoma: summary of a seven-year experience. Ann Surg 1974; 180:635–643.
12. Morton DL, Eilber FR, Holmes EC, Ramming KP. Preliminary results of a randomized trial of adjuvant immunotherapy in patients with malignant melanoma who have lymph node metastases. Aust N Z J Surg 1978; 48:49–52.
13. Wong JH, Cagle LA, Morton DL. Surgical treatment of lymph nodes with metastatic melanoma from unknown primary site. Arch Surg 1987; 122:1380–1383.
14. Sappey MPC. Anatomie, physiologie, pathologie des vaisseaux lymphatiques considérés chez l'homme et les vertebres, 4th ed. Paris: A Delahaye et Lecrosnier, 1888.
15. Robinson DS, Sample WF, Fee HJ, et al. Regional lymphatic drainage in primary malignant melanoma of the trunk determined by colloidal gold scanning. Surg Forum 1977; 28:147–148.
16. Fee HJ, Robinson DS, Sample WF, et al. The determination of lymph shed by colloidal gold scanning patients with malignant melanoma: a preliminary study. Surgery 1978; 84:626–632.
17. Holmes EC, Moseley HS, Morton DL, et al. A rational approach to the surgical management of melanoma. Ann Surg 1977; 186:481–490.
18. Rees WV, Robinson DS, Holmes EC, Morton DL. Altered lymphatic

drainage following lymphadenectomy. Cancer 1980; 45:3045–3059.
19. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53:457.
20. Kalbfleisch JD, Prentice RL. The Statistical Analysis of Failure Time Data. New York: Wiley & Sons, 1980.
21. Cox DR. Regression models and life tables. J R Stat Soc 1972; 34: 187.
23. SAS Institute. SUGI Supplemental Library User's Guide. Cary, NC: SAS Institute, 1986.
24. Hoon, DSB, Bowker R, Cochran AJ. Suppressor cell activity in human melanoma draining lymph nodes. Cancer Res 1987; 47: 1529–1533.
25. Cochran AJ, Lana AMA, Wen D-R. Histomorphometry in the assessment of prognosis in stage II malignant melanoma. Am J Surg Pathol 1989; 13:600–604.
26. Cochran AJ, Wen D-R, Morton DL. Occult tumor cells in the lymph nodes of patients with pathological stage I malignant melanoma. Am J Surg Pathol 1988; 12(8):612–618.
27. Reintgen DS, Cox EB, McCarty KS Jr, et al. Efficacy of elective lymph node dissection in patients with intermediate thickness primary melanoma. Ann Surg 1983; 198:379–385.
28. Balch CM. The role of elective lymph node dissection in melanoma: rationale, results and controversies. J Clin Oncol 1988; 6:163–172.
29. Sim FH, Taylor WF, Ivins JC, et al. A prospective randomized study of the efficacy of routine elective lymphadenectomy in management of malignant melanoma: preliminary results. Cancer 1978; 41:948–956.
30. Veronesi U, Adamus J, Bandiera DC, et al. Inefficacy of immediate node dissection in stage I melanoma of the limbs. N Engl J Med 1977; 297:627–630.
31. Veronesi U, Adamus J, Bandiera DC, et al. Delayed regional lymph node dissection in stage I melanoma of the skin of the lower extremities. Cancer 1982; 49:2420–2430.
32. Day CL, Lew RA. Malignant melanoma prognostic factors: elective lymph node dissection. J Dermatol Surg Oncol 1985; 11:233–239.
33. Eilber FR, Morton DL. Demonstration in sarcoma patients of antitumor antibodies which fix only human complement. Nature 1970; 225:1137–1138.
34. Morton DL, Wen D-R, Wong JH, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. Arch Surg (in press).
35. Wong JH, Xu S, Gupta RK. Tumor-associated antigen immune complexes. Arch Surg 1990; 125:187–191.

DISCUSSION

DR. JOHN DALY (Philadelphia, Pennsylvania): Thank you, Dr. Thompson. Malignant melanoma is a disease that is increasing in frequency in the United States of America. But fortunately it appears as though both earlier stages and thinner lesions are being identified.

Dr. Morton and his group should be complimented on developing this mathematical model that may predict survival in patients with pathologically involved regional lymph nodes. Interestingly despite the fact that these are cases that already have metastatic disease, they have noted that characteristics of the primary, such as thickness, and patient characteristics such as gender, and the site of location of the primary continue to be important survival predictors. This probably explains the tremendous heterogeneity of survivorship in this series, from 76% to 14%.

He and his group also should be complimented on their use of node mapping, because it is a major contribution, and I think it will allow us to test various concepts of elective nodal dissection with much less morbidity.

I have several questions. In the manuscript you noted that the 5-year survivorship of patients in this series was 46%, compared with an earlier series of 37%. Is this due to a more biologically favorable group of patients such as we have seen with colon cancer, for example? Or is there some other cause? It did not appear as though adjuvant immunotherapy was borne out to be effective by the univariate analysis.

Second is there any explanation as to why the site of location continues to be a major predictor of survival, even though we are talking about cases that have already metastasized to regional lymph nodes?

Third you noted that synchronous and nonsynchronous metastases are not different, but is time to the development of nodal metastasis important? In the patient who develops nodal mets 3, 4, or 5 years after the primary has been resected, do they have a better survival?

Finally have you had the opportunity to prospectively test your model in a group of patients that were not actually included in the model that you developed?

DR. G. ARANHA (Maywood): Does your scan identify those patients with intransit metastases? If it does, would removal of these metastases at the time of simultaneous node dissection and wide excision of the primary further improve your survival rates?

DR. J. B. AUST (San Antonio, Texas): This is a continuing report by Dr. Morton's group, who have long tilled in the vineyard of malignant melanoma, the chameleon of malignancies, with its bizarre course from patient to patient.

They present a very large database, and extensive statistical analysis of risk factors leading to a mathematical formula that forecasts prognostic risks. I would not touch that formula with a 12-foot pole. It is probably statistically sound, but it looks very difficult to apply.

The previous study had a poorer prognosis than the present study, and one worries that retrospective data may not forecast the future results of the next series of patients. There may be other factors, however, that account for the current better results.

One, the disease may have changed. I really doubt that has occurred. We see more patients and we see them earlier, but I do not think the disease itself has changed.

Two, patients may be getting better treatment, because we are seeing the patients earlier and operating earlier on them. It is certainly not because of effective immunotherapy or chemotherapy, because these modalities have not proved to be of significant benefit in malignant melanoma.

The third factor is probably the most important one, better classification. We may be moving a number of previous early stage cases into a more advanced stage. Dr. Morton's techniques of mapping and perhaps better pathologic examination of excised lymph nodes all tend to produce more lymph-node–positive cases than there were in the previous group, reducing the number of stage I cases and increasing the number of stage II with patients of lesser tumor burden, thereby improving the reported results of both stages.

So I think that what may be happening is that they are improving the final results by restaging the patients in a more effective way, and not because of better therapy.

I would like Dr. Morton to address that particular issue, and I wish him well in his continued studies. He has been one of the senior investigators of malignant melanoma over a number of years.

DR. EDWARD KREMENTZ (New Orleans, Louisiana): President Thompson, I take this occasion to rise to discuss Dr. Morton's report of his vast experience with melanoma in southern California. With the rising rates of melanoma in this country, he will soon be able to report more cases than the Australians.

I am concerned about the present tendency for surgeons to discount the good effects of chemotherapy, and particularly those obtained by regional chemotherapy. When I started in practice over 40 years ago, just about all of the solid tumors treated with chemotherapy were treated by surgeons. Now with the increasing numbers of medical oncologists, this case material is largely being referred to that group. Even in the field of regional chemotherapy, we are losing some of these patients to the interventional radiologists working with the medical oncologists.

Our group has treated over a thousand patients with limb melanomas by chemotherapy and by regional perfusion. Although we cannot match the numbers of cases that Dr. Morton has presented, 1134 cases with regional lymph node dissection, we have had 129 patients with limb melanoma with positive lymph nodes treated by perfusion and regional lymph node dissection.

Our long-term cumulative survival rates at 5, 10, 15 years, are 52%, 51%, and 49%, respectively, which are better than Dr. Morton's reported rates for node dissection alone.

I would like to ask Dr. Morton, what is his therapeutic approach to melanoma patients with stage 3-A, satellitosis, or stage 3-AB disease, satellites and positive nodes? We find that with intransit disease excision alone, or systemic chemotherapy or immune therapy, do not provide long-term control. With intransit disease, our 10-year survival with chemotherapy by perfusion is 23%. If the satellites can be resected, it increases to 39%. With 3-AB disease, perfusion, and regional node dissection, the survival rate 28%.

There is one interesting point on which I would like to ask Dr. Morton to comment. When we did lymph node dissections for primary melanoma with positive lymph nodes, our 15-year survival rate was 30%, but if we had patients who had had previous resection of the primary disease, who showed up later with positive lymph nodes, perfusion and regional node dissection at that time produced a survival rate of 51%. I suppose the better survival had to do with a more favorable slowly growing type of melanoma, but I would like to hear his comments on this observation.

DR. BLAKE CADY (Boston, Massachusetts): This report continues the tradition of innovation that Don has established at UCLA, with over 5000 cases in 18 years and a 22% incidence of positive nodes; a 46% overall 5-year survival, which is clearly better, as he pointed out, than we are used to; and 41% survival at 10 years. Seven hundred thirty-seven patients form the basis of this report on the patients with positive nodes, which is 14% of the entire series but only about 65% of the cases with positive nodes, and I just wonder what type of subtle selection factors have gone into this subgroup of patients who had complete data.

There are some expected findings, such as the fact that there is no outcome difference with immunotherapy and no outcome difference, in my interpretation, from the time of the primary to death between immediate and delayed node dissection. There is no difference in survival based on the mathematical model, which you would not expect, because the mathematical model is derived directly from the data, and so when you reapply the model to the data you would not expect anything different; the real test of this model would be when it is applied prospectively.

There are some unexpected findings, however. There is no outcome difference in the number of positive nodes in melanomas between 0.76 and 4 mm in thickness in some selective groups. There is no difference in the incidence of positive nodes all the way from 0.76 to 4 mm in thickness, which is certainly different from what we have been led to believe and argues for selection bias in the analyzed group.

That unknown primaries behave like thin melanomas is another interesting aspect of his report. Twenty per cent of the clinical stage I had micrometastases, and about 20% of the clinical stage II had macrometastases, which would imply that all micrometastases eventually become macrometastases, quite different from breast cancer in the NSABP trials about untreated node metastases. Most remarkable of all is the selective low-risk group with positive nodes who have between 70% and 80% survival long-term with positive nodes. That is pretty remarkable, and it would be nice to be able to define that group very precisely.

I calculated, in trying to juggle with the figures, about a 3% overall impact on survival comparing prophylactic *versus* therapeutic node dissection, but it would be nice to know which selective risk group achieves that benefit. Is it the high-risk group or the low-risk group? I also calculated that the mortality rate in these patients is about 16% in the first year, 18% in the second year, 11% in the third year, 6% in the fourth year, 3% in the fifth year, 1%, as he noted, between 5 and 10 years, and about 0.6% yearly between 10 and 15 years. But is that mortality rate different with only micrometastases? And is this just another example of lead-time bias?

How many patients had deep groin nodes positive? And is that part of the regional nodal dissection policy? Do all such patients die and, therefore, deep nodes do not need to be removed? What is it about men that the number of positive nodes does not have an impact, and in women, why is it that the depth does not have any impact on survival when the nodes are positive?

Development of risk groups is critical in a sophisticated analysis of prognoses. Of course it only really helps if we have something to add in adjuvant therapy, and we do not have that in melanoma, but it would certainly be useful to help us make selections about who gets prophylactic node resection and who does not. There is an innovative technique that Don has developed of picking up the sentinel node, which will help us decide which nodes to dissect.

Thank you very much for asking me to discuss this and seeing the interesting data.

DR. E. COPELAND (Gainesville, Florida): Of the material presented, the so-called blue dye technique would be most likely to change my method of managing patients with melanoma, because the remaining data confirm my current treatment policies.

I was surprised, however, that there were no false-negatives, in other words, if the blue dye went to the "sentinel node" and it was negative, then no other regional lymph nodes contained metastatic melanoma. This conclusion would mean that no "skip metastases" existed in your dissected regional node specimens—a hard conclusion for me to accept because skip metastases in regional nodes have been noted for other surface malignancies such as breast cancer.

Explain to us why you think there were no false-negatives in your series.

DR. DONALD L. MORTON (Closing discussion): Dr. Daly wants to know why the survival is better in this series, 46% of 5-year *versus* our earlier series at 37%. We really do not have a good explanation for that. We thought it was because of immunotherapy, as there is about a 5% to 10% improvement at all points in the patients who got immunotherapy *versus* those who did not.

By univariate analysis, however, the differences were not statistically significant. And as we looked into the various heterogenous subsets, it is very clear that this type of comparison has to be very carefully done because of the huge difference in survival in the various subsets of patients.

Why is there a difference between the extremity and nonextremity sites in terms of the significance of numbers of involved nodes and the improved survival in women? I do not have an explanation for that. I think the fact that the unknown primaries paralleled the thin melanomas might be explained by the fact that if you have a thin melanoma, the host is more likely to reject it, and so that would not be surprising.

As Dr. Daly and several discussants brought up, the real test of our model is to apply it prospectively to our own series, and to other series, and we are now going about that. We have 2 years of patients currently being tested, and we hope to collaborate with those of you that have large data sets to compare survival in your series with that in ours.

The explanation for synchronous *versus* asynchronous metastasis and difference in survival is another point on which several people commented. And of course as you know, although about 85% of the patients who are going to develop lymph node metastasis from melanoma do so within the first 3 years, there is quite a tail of people that will go 10 and even 20 years before they develop clinical evidence of regional metastases. That probably indicates a tumor–host relationship that is much more favorable than that in patients who develop early metastasis. That may be one of the reasons for the improved survival of people with asynchronous metastasis in some series.

Dr. Aranha asked, will this technique identify intransit metastasis? And the answer is: It will not.

Dr. Aust has asked, again, why the improved survival? It is certainly true that some of this may be due to the fact that more of the patients in this series had elected no dissection. If we just look at those that have no dissections for clinically positive nodes, however, we have a 51% 5-year survival in that group compared with 37% in our prior series. And we cannot say that is the explanation.

Surprisingly if we look at all of our melanoma patients, and we are seeing about 400 a year now, the median depth of thickness of all of our patients has been remarkably constant over the last 20 years. And I can only assume that that is a result of the selection that occurs in the patients that are sent to us. The very thin early lesions are being treated in the local community hospitals, and they never find their way to a tertiary referral center such as ours.

Dr. Krementz has pointed out the extremely excellent results in people that have had limb perfusions and wants to know what our treatment of people with satellite disease is, and at present initially we treat with

90% of patients, and gives us a 25% 5-year survival. And in those that we cannot control with interlesional BCG, we perform a heat perfusion just as you, and we control 75% of those with perfusion.

So we have confirmed completely your experience with the effectiveness of heat perfusion in the management of this disease, but we have saved that for patients whom we cannot control with interlesional BCG.

Is the statistical model different from the group as a whole? The answer is that survival is exactly the same in these two sets of patients. The problem is that often the original primary lesion was not available for us for those 400 patients that we did not have complete data on.

Regarding the deep groin nodes, we have previously reported that patients' clinically negative but microscopically positive inguinal nodes have deep node dissections. They have the same survival at 5 years when deep iliac nodes are involved as do people who just have inguinal nodes involved. Therefore we still do a deep node dissection. In those that had clinically positive inguinal nodes, however, the 5-year survival of those have deep iliac nodes involved drops off dramatically. It is only about 15%.

Dr. Copeland asked, is it really possible that this technique has no false-negatives? The false-negative that we observed early on was due to the fact that we were not adequately injecting the dye. One must continue injecting the dye, because it goes right through the first echelon node to the second echelon node. If one does not keep injecting the dye every 20 minutes, the second node may be thought to be the sentinel node.

Once we learned that, we have not had any false-negatives. Now that is determined by two ways. Number one, all of the nodes in the lymphadenectomy specimen were examined both by standard hematoxylin and eosin (H & E) and immunohistochemical staining with S-100 and monoclonal antibodies. We did not find skip metastasis in melanoma. I think that melanoma is not the same as breast cancer. It is different.

Secondly, in the last 2½ years we have been doing just the sentinel lymphadenectomy. We have had no recurrences in a groin or axilla or neck when the sentinel node was negative. Although we need a little longer follow-up to be certain, it is encouraging that none of our sentinel-node–negative patients has developed a recurrence.

Inhibition of Macrophage-activating Cytokines is Beneficial in the Acute Septic Response

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Interferon-γ and other cytokines enhance macrophage (MØ) antimicrobial function and have been considered for therapeutic use in sepsis. Systemic sequelae of macrophage activation, however, are unclear. This study examined the effects of MØ activating cytokines (interferon-γ [IFN-γ] and interleukin-4 [IL-4]) and monoclonal antibodies directed against these cytokines in modulating the acute septic response. CFW/Swiss Webster mice (n = 345) received endotoxin (lipopolysaccharide [LPS]: 60 mg/kg body weight intraperitoneally) and were randomized to five treatment groups: IFN-γ (10^4 units), IL-4 (10^4 units), IgG_1 isotype antibody (TRFK5: 200 μg), anti–IFN-γ (200 μg), or anti–IL-4 (200 μg) monoclonal antibodies (MAbs) given simultaneously or 2 hours after LPS. Animals were divided into two groups and studied for mortality or measurment of peritoneal MØ superoxide anion release (O_2^-), tumor necrosis factor (TNF), and IL-6 production 6 hours after administration of LPS ± experimental regimens. Serum TNF and IL-6 also were assessed at 2 and 4 hours after LPS, respectively. Administration of LPS resulted in a 27% survival compared with 10% in the IFN-γ and 13% in the IL-4 groups. Treatment with anti–IFN-γ offered protection against LPS lethality (93%–100% survival, $p < 0.001$ *vs.* other groups) when given either simultaneously or 2 hours after LPS. Anti–IFN-γ also significantly decreased PMØ O_2^- and TNF release. Thus anti–IFN-γ may have an important role in the modulation of the acute septic response.

BACTERIAL INFECTION REMAINS a common cause of death among critically ill, hospitalized patients despite numerous therapeutic advances.[1,2] Several studies have reported an increased incidence of gram-negative septicemia with an associated mortality rate of 20% to 35%.[3,4] In those patients who develop gram-negative septic shock, the mortality rate may be as high as 50% to 80%.[5] Recent attention has focused on the pathogenesis of gram-negative sepsis and, in particular, the toxic role of endotoxin or lipopolysaccharide (LPS), which is a component of the outer membrane of gram-negative and some gram-positive bacteria. Many of the effects of LPS are mediated through the release of cytokines, including tumor necrosis factor (TNF), interleukin-1 (IL-1), and interleukin-6 (IL-6). Elevated serum TNF levels occur during experimental endotoxemia in humans, primates, and canines[6,7] and have been observed in a number of infectious disease states.[8–10] Furthermore direct intravenous infusion of TNF induces effects mimicking septic shock.[11] Moreover in experimental studies passive immunization with anti-TNF monoclonal antibody (MAb) is protective against the development of septic shock and its sequelae.[12]

Although functionally not as well defined, IL-1 appears to act as a potentiator of TNF activity in rabbits[13] and mice,[14] and high levels of IL-6 have been identified in malaria and meningococcal meningitis.[9] Monoclonal antibodies directed against IL-6 have recently been shown to be beneficial when given simultaneously with *Escherichia coli* or TNF administration,[15] and an IL-1 receptor (IL-1R) antagonist has proved effective in preventing lethal *E. coli*-induced shock in rabbits and baboons.[16–18]

Central to the production and release of these cytokines is the mononuclear phagocyte, which includes both peripheral blood monocytes and fixed tissue macrophages (MØ). Macrophage activation results in increased release of these cytokines as well as reactive oxygen intermediates. As shown in Figure 1, recent studies have used MAbs against these cytokines to alter the acute septic response.

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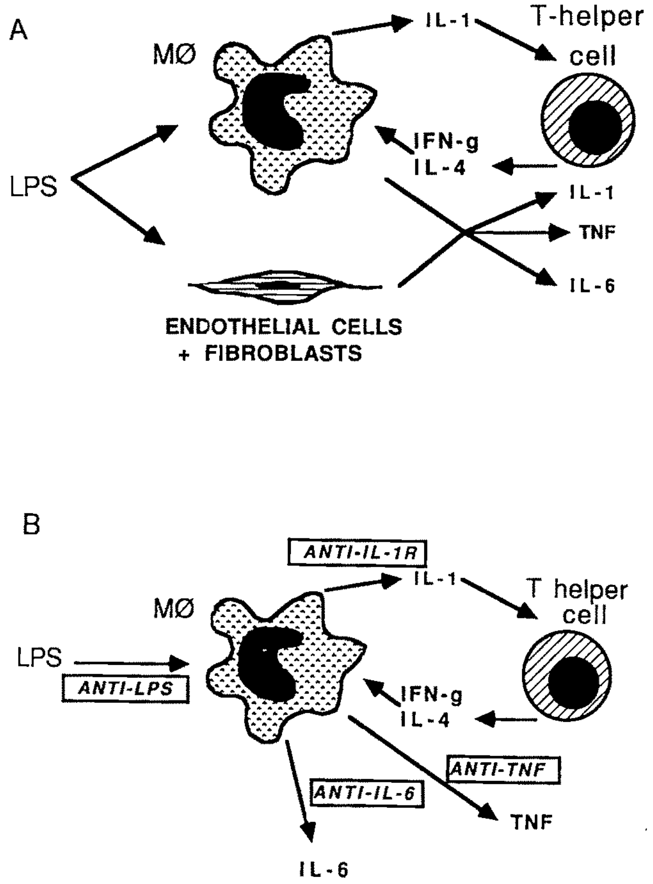

FIGS. 1A and 1B. (A) Monoclonal antibodies have been developed and used against endotoxin (LPS), the macrophage proinflammatory cytokines TNF and IL-6, and IL-1R. (B) Proposed steps in the activation of macrophages by endotoxin. Macrophage–endotoxin interaction may lead to IL-1 release, which in turn, stimulates T-helper cell subsets, with subsequent release of the macrophage-activating factors, interferon-γ and IL-4. These factors, in turn, activate macrophages for release of TNF and possibly IL-6. Endothelial cells, epithelial cells, and fibroblasts may also secrete proinflammatory cytokines when appropriately stimulated.

Macrophage activation is induced *in vivo* and *in vitro* by interferon-γ (IFN-γ)[19] and *in vitro* by interleukin-4 (IL-4)[20,21] (Fig. 1A). Elevation in serum IFN-γ levels occurs during the course of septic shock.[5] It is reasonable to postulate that IFN-γ, as well as other MØ-activating cytokines such as IL-4, may have a role in modulating the acute septic response because of their potent macrophage-activating capacity.

These studies were designed to assess the role of macrophage activation during the acute septic response and, in particular, the potential benefit of monoclonal antibodies directed against the macrophage-activating factors IFN-γ and IL-4 (Fig. 1B).

Methods

Mice

Three hundred forty-five virus-free, female Swiss-Webster (CFW) mice (Charles River Laboratories, Wilmington, MA), 6 to 8 weeks old, were studied. After a 7-day period of acclimation on a regular diet, animals were weighed and randomized for either *in vivo* mortality analyses or *in vitro* studies (Fig. 2).

Reagents

Endotoxin (LPS) from *E. coli* serotype 055.B5, 3[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), phorbol myristate acetate, and superoxide dismutase were purchased from Sigma Chemical Co. (St. Louis, MO).

Cytokines

Murine rIFN-γ and rIL-6 were purchased from Genzyme Corp, (Cambridge, MA), specific activity: 4.5 to 9 $\times 10^6$ μm/mg. Murine rIL-4 was provided by Immunex (Seattle, WA), specific activity: 10.5×10^6 μm/mg. Murine rTNFα was kindly donated by Dr. L. Moldower (Cornell University, New York, NY).

Monoclonal Antibodies

The rat IgG1 antimurine IFN-γ (R4-6A2) was purchased from ATCC (Rockville, MD), grown in culture, and purified over a protein G column (Pharmacia, Piscataway, NJ). The 11B11 (anti-IL-4) also was purchased from the ATCC, grown in culture, and purified over a protein G column. The TRFK5 (anti–IL-5) hybridoma, used as a control antibody, was a gift of Dr. R. L. Coffman (DNAX, Palo Alto, CA). Monoclonal antibodies and cytokines were tested for the presence of LPS using the Limulus Assay (Sigma Chemical Co, St. Louis, MO) and found to contain less than 0.4 ng/mL LPS per milliliter of antibody. Monoclonal antibodies and cytokines were administered intravenously in LPS-free phosphate-buffered saline (PBS: 200 μL/mouse)

In Vivo *Mortality Studies*

Mice (n = 15/group) were randomized to receive LPS (60 mg/kg body weight [BW] intraperitoneally [I.P.]) immediately followed by a treatment regimen as outlined in Figure 1. Survival was assessed daily up to 5 days, after which time no mortality was noted in these studies.

In a second experiment, mice (n = 15/group) received LPS (60 mg/kg BW I.P.) Two hours later, animals were randomized to a treatment group as outlined in Figure 1. Survival was assessed daily up to 5 days.

Isolation and Assays of Peritoneal Macrophages

Peritoneal macrophages (PMØ) were harvested by peritoneal lavage with sterile PBS, centrifuged, washed with PBS, and resuspended in Krebs Ringer's phosphate dextrose (KRPD).[22] Cells were plated at a concentration

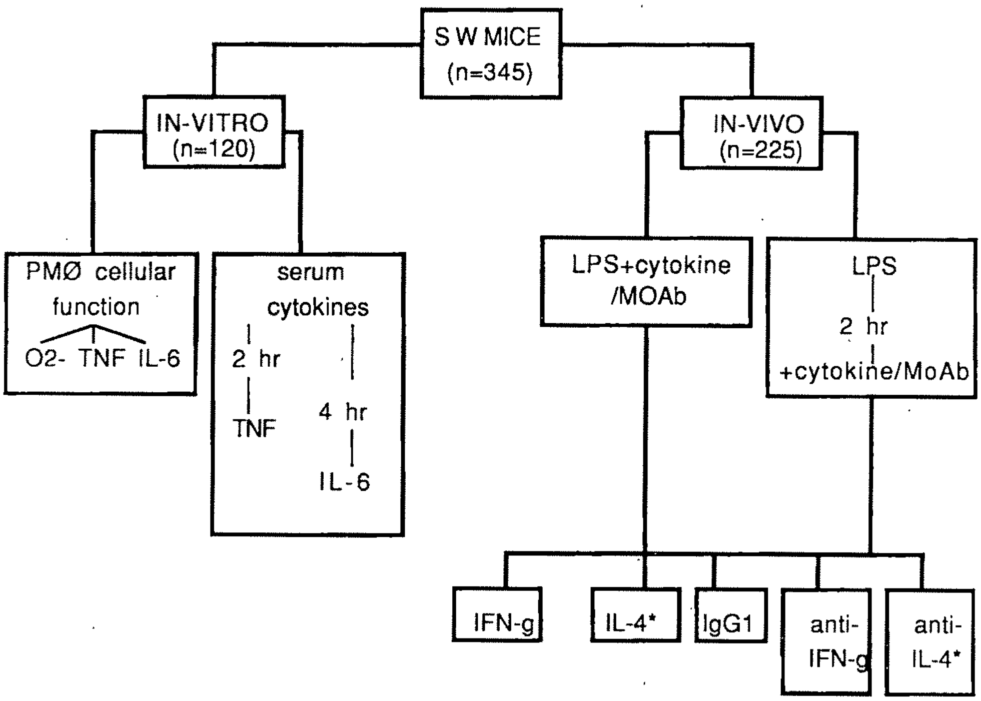

FIG. 2. This flow diagram highlights the different *in vitro* and *in vivo* groups used in this study. *Not studied in delayed (2-hr) treatment group.

of 1 × 10^6 cells/mL, allowed to adhere for 2 hours at 37 C, washed twice in PBS to remove nonadherent cells, and studied for superoxide anion production, TNF, and IL-6 release.

Preparation of Serum Samples

Blood samples for determination of serum cytokines were obtained by cardiac puncture of mice after CO_2 asphyxiation. Mice (n = 3/group), were randomized to receive a specific treatment regimen, followed immediately by LPS administration (60 mg/kg BW I.P.), and were killed at either 2 or 4 hours for TNF and IL-6 estimation, respectively. Serum samples were obtained from pooled blood and stored at −70 C until assays were performed.

Assay for Superoxide Anion Generation

O_2^- generation was measured as the superoxide dismutase-inhibitable reduction of ferricytochrome C in response to a stimulus of phorbol myristate acetate (PMA 1 μg/mL). Results are expressed as nmol O_2^-/mg protein/90 minutes. Protein content of adherent cells was determined by Lowry's method.[23]

Tumor Necrosis Factor Bioassay

The tumor necrosis factor assay used L929 mouse fibroblasts, which were grown to confluency in a 75-cm^2 flask. L929 cells were plated at 5 × 10^5 cells/well for samples and standard curve analysis. After 24 hours all wells were aspirated and replaced with 80 μL actinomycin D and complete media. Samples and standards then were added in a 20-μL volume, and incubated for 18 hours. For cellular TNF release, MØ were stimulated with LPS (1 μg/mL) for 2 hours and samples were stored at −70 C until used. 3[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT: 10 μL/well) was then added to each well and incubation was continued for 4 hours. All wells then were aspirated and 100 μL isopropanol and 0.04 N HCl were added. The plate then was incubated for a further 10 minutes and 100 μL distilled water was added per well. Absorbance was read with an enzyme-linked immunosorbent assay (ELISA) plate reader (Molecular devices, Menlo Park, CA) at 570 nm with 690 nm subtraction.

Interleukin-6 Bioassay

Interleukin-6 was assayed according to the technique of Aarden et al.[24] Briefly 5 × 10^3 − 1 × 10^4 B9 cells (subclone of B13.29 kindly provided by Dr L. Moldower) were cultured in 96-well flat-bottomed microtiter plates in 200 μL complete RPMI. For cellular IL-6 release, MØ were stimulated with LPS (100 ng/ml) for 12 hours and samples were stored at −70 C until used. Freshly thawed samples were assayed in duplicate and added to the cells at 1:5, 1:25, 1:125, 1:625 dilutions. Sixty hours later 10 μL Dulbecco's modified Eagle's medium containing 6 mg/mL MTT was added to each well and incubated for 4 hours. The assay was completed as described for the TNF assay. One unit of IL-6 was defined as the concentration required for half-maximal MTT incorporation. Murine rIL-6 (Genzyme Corp Cambridge MA) was used as the standard. Neither rIL-1-alpha nor rTNF induce B9 growth under these experimental conditions.

Statistical Analysis

Superoxide anion generation, cellular TNF, and IL-6 were performed on pooled MØ, using at least five mice per assay. Each experiment was performed three to five times. Serum cytokine analysis was performed on pooled serum, using three to five mice per assay. *In vivo* mortality studies used at least 15 mice per group. Statistical analysis was performed using the unpaired Student's t test for comparison of two groups and ANOVA for multiple comparison of means of several groups. The chi square analysis was used to assess survival in LPS studies. The level of significance was determined at $p < 0.05$. Data are presented as the mean ± standard deviation.

Results

Superoxide Anion Generation

As shown in Figure 3, endotoxin administration primed PMØ *in vivo* for enhanced O_2^- release. Interferon-γ synergized with LPS to induce the highest state of macrophage activation as reflected in mean O_2^- production compared with all other groups ($p < 0.05$). Both anti–IFN-γ and anti–IL-4 inhibited LPS-induced priming for O_2^- release, indicating their potential to inhibit macrophage activation.

Peritoneal Macrophage TNF Release

As shown in Figure 4, IFN-γ and IL-4 were associated with significantly elevated mean cellular TNF release compared with the control group ($p < 0.05$) (Fig. 4). Anti–IFN-γ significantly inhibited mean cellular TNF release from PMØ compared with the isotype control ($p < 0.01$). Anti–IL-4 failed to downregulate *in vivo* LPS-induced mean cellular TNF release.

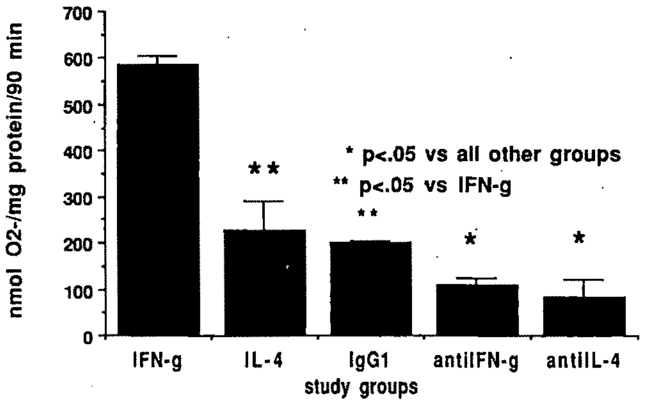

FIG. 3. Bar graph of superoxide anion production (O_2-) by peritoneal macrophages from each of the study groups (n = 3–5/group) after *in vivo* priming with LPS (60 mg/kg) for 6 hours and *in vitro* stimulation with PMA (1 μg/ml) for 90 min, after 2 hours of macrophage adherence.

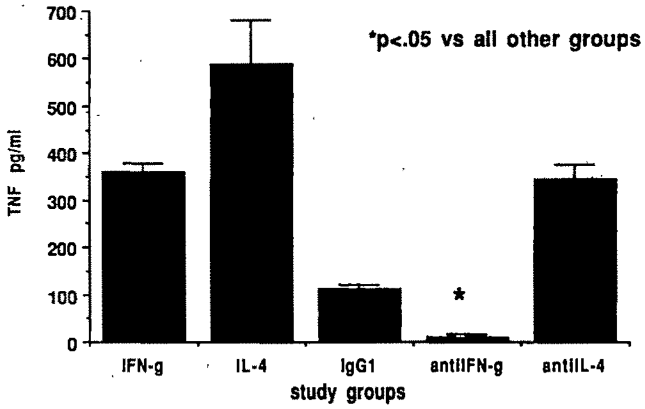

FIG. 4. Bar graph of TNF release by peritoneal macrophages (mean ± SD) from each of the study groups (n = 3–5 mice/group) after *in vivo* LPS challenge (60 mg/kg) for 6 hours, and *in vitro* LPS stimulation (1 μg/ml) for 2 hours, after 2 hours of macrophage adherence.

Serum TNF Levels

As shown in Figure 5, IFN-γ administered simultaneously with LPS was associated with significantly higher mean serum TNF levels compared with all other groups ($p < 0.05$), indicating an apparent synergy with LPS (Fig. 5). Lowest levels of mean serum TNF were found in mice treated with the isotype antibody.

Peritoneal Macrophage IL-6 Release and Serum IL-6 Levels

Mean LPS-induced macrophage IL-6 release was not significantly different among study groups (Fig. 6). The highest mean serum IL-6 levels, however, were found in IFN-γ and IL-4 treatment groups (Fig. 7). Treatment with anti–IL-4 was associated with significantly lower mean serum IL-6 levels compared with all other groups ($p < 0.01$).

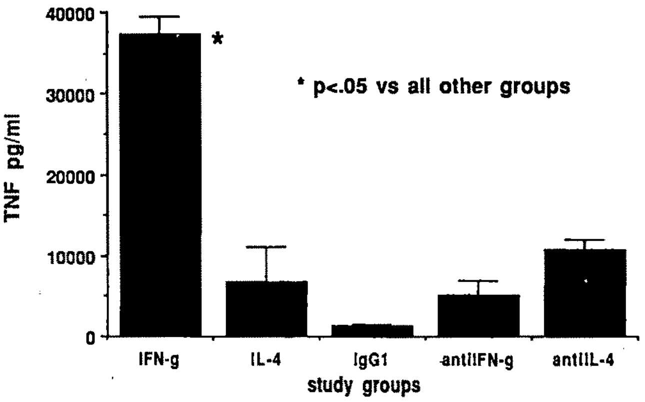

FIG. 5. Graph showing serum TNF levels (mean ± SD) for each study group. Serum was harvested (n = 3 mice/group) 2 hours after simultaneous LPS challenge (60 mg/kg) and cytokine/monoclonal antibody administration.

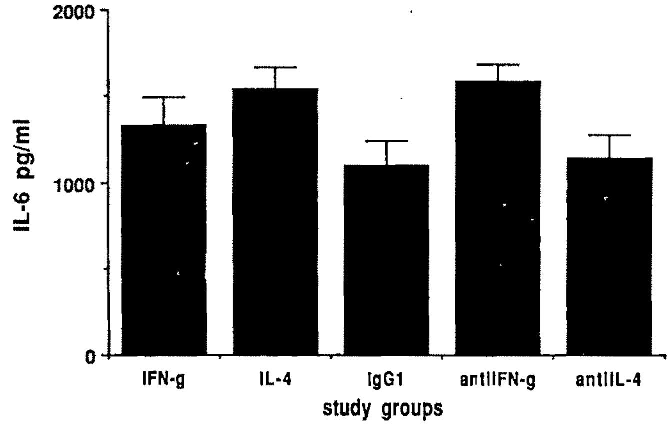

FIG. 6. Bar graph of IL-6 release by peritoneal macrophages (mean ± SD) from each of the study groups (n = 3–5 mice/group) after *in vivo* LPS challenge (60 mg/kg) for 6 hours, and *in vitro* LPS stimulation (100 ng/ml) for 12 hours, after 2 hours of macrophage adherence.

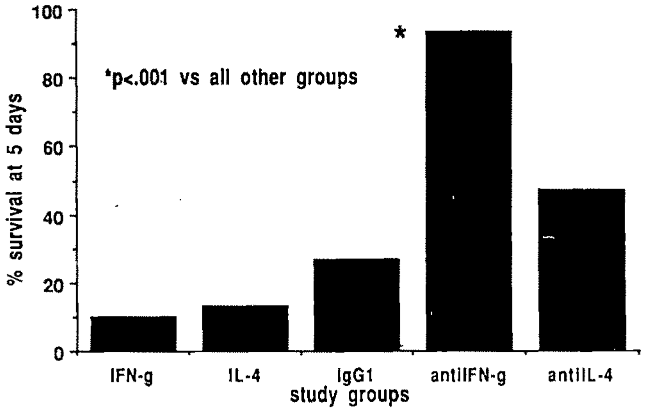

FIG. 8. Bar graph of survival after 5 days in each of the study groups after simultaneous LPS challenge (60 mg/kg) and cytokine/monoclonal antibody administration.

Simultaneous LPS Administration and Cytokine/MAb Administration

Endotoxin administration was associated with only a 27% survival in the control MAb study group (Fig. 8). Simultaneous administration of either IFN-γ or IL-4 with LPS resulted in less survival (10% and 13%) compared with the control group. Anti–IFN-γ treatment significantly enhanced survival to 93% compared with results in all other groups ($p < 0.001$). Anti–IL-4 conferred only a slight survival advantage (27% *vs.* 47%) compared with the control isotype antibody.

LPS and Delayed (2-hour) IFN-γ/MAb Administration

Administration of the IgG_1 isotype control antibody 2 hours after LPS challenge was associated with a survival rate of 33%, which was similar to that obtained when this antibody was administered simultaneously with LPS (Fig. 9). Despite a 2-hour treatment delay, anti–IFN-γ conferred a significant survival advantage (100%), compared with either IFN-γ (47%) or the control IgG_1 antibody (33%). Administration of IFN-γ 2 hours after LPS was less lethal than simultaneous injection (47% *vs.* 10%)

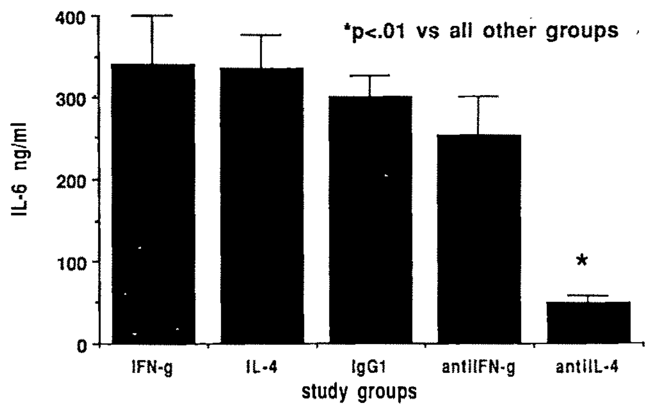

FIG. 7. Graph showing serum IL-6 levels (mean ± SD) for each study group. Serum was harvested (n = 3 mice/group) 4 hours after simultaneous LPS challenge (60 mg/kg) and cytokine/monoclonal antibody administration.

Discussion

The pathophysiology of the septic response to endotoxemia and bacterial infection is based in part on the inflammatory mediators released by MØ in various states of activation. Tumor necrosis factor, IL-1, IL-6, platelet-activating factor, complement, eicosanoids, and reactive oxygen intermediates all have been implicated in inducing the deleterious effects that are associated with the host's acute septic response. Aggressive use of antimicrobial agents may combat bacteremia, but often falls short of ameliorating adverse responses of the host during sepsis. Use of monoclonal antibodies specifically directed against key inflammatory mediators, including LPS, TNF, IL-6, and IL-1R has proven to be a major development in therapy aimed at modulating the acute septic response.[12,15,16,25] This study provides evidence that endotoxemia is associated with MØ activation, and that administration of cytokines with *in vitro* MØ activating properties (IFN-γ and IL-4), in association with LPS results in even higher states of macrophage activation, as evidenced by significantly higher peritoneal macrophage mean O_2^- generation, mean TNF release, and mean serum TNF levels (IFN-γ only). Administration of monoclonal antibodies aimed at specifically blocking each of these MØ activating factors was beneficial in inhibiting release of one or more of these cytokines, indicating the importance of IFN-γ and IL-4 as MØ activating cytokines during endotoxemia. Furthermore, anti–IFN-γ significantly enhanced survival from lethal endotoxin challenge through modulation of MØ activation state.

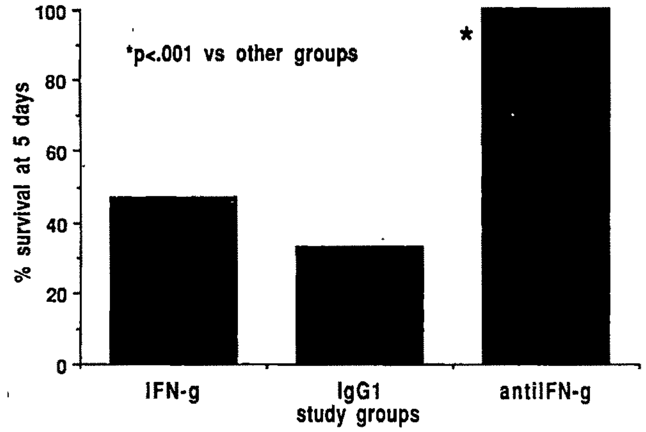

FIG. 9. Bar graph of survival 5 days after LPS challenge (60 mg/kg) with delayed treatment (2 hours) administration, consisting of either interferon-γ, IgG1 isotype control, or anti-interferon-γ study groups.

Interferon-γ is a T cell lymphokine, released during MØ–T-cell interaction. It has a broad range of functions and is primarily recognized for its potent capacity to activate MØ for enhanced microbicidal activity, including oxygen free radical production, phagocytosis, and killing of intracellular pathogens.[26] A number of studies have examined the potential benefit of IFN-γ administration in various disease states. In a rodent model of hemorrhagic shock, Livingston and Melangoni[27] demonstrated a reduction in abscess size and bacterial count using a combination of IFN-γ and cefamandole, indicating the ability of IFN-γ to restore host immune function. Hershmann et al.[28] showed that IFN-γ was beneficial in a model simulating bacterial infection after trauma. Subsequent studies from this laboratory, however, demonstrated a detrimental effect when IFN-γ treatment was instigated concomitant with cecal ligation and puncture.[29] Heremans et al.[30] have analyzed the endogenous role of IFN-γ in the generalized Shwartzman reaction. A good correlation was found between levels of interferon and mortality. Treatment with IFN-γ rendered mice more susceptible to the reaction, and anti–IFN-γ administration was protective. Heinzel[31] has demonstrated a deleterious role for IFN-γ in the pathogenesis of experimental endotoxemia.

In this study administration of IFN-γ was not protective during endotoxemia whether given simultaneously or 2 hours after the administration of LPS. Moreover IFN-γ was associated with a higher mortality when given at the same time as LPS, compared with the control group (10% *vs.* 27%). Interestingly this deleterious effect was not seen when IFN-γ treatment was delayed for 2 hours after LPS administration. Administration of anti–IFN-γ was highly protective after lethal endotoxin challenge, whether given at the same time as LPS or as a delayed treatment regimen. These findings indicate that although IFN-γ may be beneficial as an immunostimulant in immunosuppressive disease states, during endotoxemia IFN-γ is a pathogenic mediator. Thus IFN-γ may have a therapeutic role when *in vivo* MØ antimicrobial function is impaired, but it may be harmful when given during the acute septic response when MØs appear to be in an activated state.

Interleukin-4 (IL-4) is a T-cell–derived glycoprotein that was originally characterized a B cell stimulatory factor-1.[32] Functions of IL-4 on human monocytes and mouse macrophages also have been reported. Interleukin-4 has been shown to increase antigen-presenting ability, surface Ia expression, and tumoricidal activity in mouse macrophages.[33,34] Preliminary studies from our laboratory indicate that IL-4 can prime MØ for enhanced release of reactive oxygen species.[21] Little is known regarding the *in vivo* antimicrobial potential of this cytokine. McBride et al.[35] failed to demonstrate a protective effect when IL-4 was administered before a lethal dose of either LPS or IL-2. We also have failed to identify any beneficial effect when this cytokine was administered concurrent with LPS. Administration of anti-IL-4, however, was associated with a slightly greater survival after LPS, compared with controls (27% *vs.* 47%) and was associated with significantly greater survival compared with the IL-4 group (13%).

The role of MØ-derived antimicrobial mediators during the acute septic response has been well documented. Previous studies have attempted to correlate serum cytokine levels with survival after sepsis. In studies comparing levels of serum cytokines, TNF appears to most closely correlate with ultimate outcome.[5,36] Serum cytokine levels do not appear to be as predictive as other physiologic parameters, however.[5] Our study attempted to correlate cellular production and serum levels of these mediators with survival. In animals treated with IFN-γ, the highest macrophage mean O_2^- production and mean serum TNF levels were identified and were associated with greatest mortality. Treatment with IL-4 also was associated with elevated mean O_2^- and significantly elevated cellular TNF release. Anti–IFN-γ significantly downregulated mean O_2^- and cellular TNF release, but not mean serum TNF levels. In contrast to TNF, mean cellular IL-6 release was similar between groups; however anti–IL-4 significantly downregulated mean serum IL-6 levels, a potential mechanism by which this monoclonal antibody may be beneficial. Overall peritoneal macrophage production of O_2^- most closely correlated with eventual outcome. These findings add further evidence to the role of the activated MØ in mediating the deleterious effects of LPS.

The consequences of septic shock remain a serious therapeutic dilemma. Treatment of the septic source, antimicrobial agents, and supportive care are vital but do not offer complete protection against the often lethal consequences of cellular immune responses to infection. Use of monoclonal antibodies aimed at modulating the degree of macrophage activation, possibly combined with antimicrobial agents, offer a novel treatment strategy in the septic host.

Acknowledgment

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References

1. Bryan CS, Reynolds KL, Brenner ER. Analysis of 1186 episodes of gram-negative bacteremia in a non-university: the effects of antimicrobial therapy. Rev Infect Dis 1983; 5:629–638.
2. Calandra T, Glauser MP. Cytokines and septic shock. Diagn Microbiol Infect Dis 1990; 13:377–381.
3. Kreger BE, Craven DE, Carling PC, Mc Cabe WR. Gram-negative bacteremia: III. Reassessment of etiology, epidemiology and ecology in 612 patients. Am J Med 1980; 68:332–343.
4. Kreger BE, Craven DE, Carling PC, McCabe WR. Gram-negative bacteremia: IV. Reevaluation of the clinical features and treatment in 612 patients. Am J Med 1980; 68:344–355.
5. Calandra T, Glauser MP, Schellekens J, et al. Treatment of septic shock with human IgG antibody to *Escherichia coli J5:* a prospective, double-blind, randomized trial. J Infect Dis 1988; 158: 312–319.
6. Beutler BA, Milsark IW, Cerami A. Cachectin/tumor necrosis factor: production, distribution and metabolic fate in vivo. J Immunol 1985; 135:3972–3977.
7. Natanson C, Eichenholz PW, Danner RL, et al. Endotoxin and tumor necrosis factor challenges in dogs simulate the cardiovascular profile of human septic shock. J Exp Med 1989; 169:823–832.
8. Waage A, Halstensen A, Espevik T. Association between tumor necrosis factor-like cytotoxicity in serum and fatal outcome in patients with meningococcal disease. Lancet 1987; 1:355–357.
9. Waage A, Braendtzaeg P, Halstensen A, et al. The complex pattern of cytokines in serum from patients with meningococcal septic shock. J Exp Med 1989; 169:333–338.
10. Giradin E, Grau GE, Dayer JM, et al. Tumor necrosis factor and interleukin-1 in the serum of children with severe infectious purpura. N Engl J Med 1988; 319:397–400.
11. Tracey KJ, Beutler B, Lowry SF, et al. Shock and tissue injury induced by recombinant human cachectin. Science 1986; 234:470–474.
12. Tracey KJ, Fong Y, Hesse DG, et al. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteremia. Nature 1987; 330:662–664.
13. Okusawa S, Gelfand JA, Ikejima T, et al. Interleukin-1 induces a shock-like state in rabbits: synergism with tumor necrosis and the effect of cyclooxygenase inhibitors. J Clin Invest 1988; 81: 1162–1172.
14. Waage A, Espevik T. Interleukin-1 potentiates the lethal effect of tumor necrosis factor α/cachectin in mice. Lancet 1988: 167: 1987–1992.
15. Starnes HF, Pearce MK, Tewari A, et al. Anti-IL-6 monoclonal antibodies protect against lethal *Escherichia coli* infection and lethal tumor necrosis-α challenge in mice. J Immunol 1990; 145: 4185–4191.
16. Ohlsson K, Bjork P, Bergenfeldt M, et al. Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. Nature 1990; 348:550–552.
17. Wakabayashi GO, Gelfand JA, Burke JF, et al. A specific receptor antagonist for interleukin 1 prevents *Escherichia coli*-induced shock in rabbits. FASEB J 1991; 5:338–343.
18. Fischer E, Marano MA, Van Zee KJ, et al. IL-1 receptor blockade attenuates the hemodynamic and metabolic consequences of lethal E. coli shock. 2nd International congress on the immune and metabolic consequences of trauma, shock and sepsis mechanisms and therapeutic approaches. Munich, Germany, March 1991.
19. Murray HW, Spitalny GW, Nathan CF. Activation of mouse peritoneal macrophages in vitro and in vivo by interferon-gamma. J Immunol 1985; 134:1619–1622.
20. Phillips WA, Croatto M, Hamilton JA. Priming the macrophage respiratory burst with IL-4: enhancement with TNFα but inhibition by IFN-g. Immunology 1990; 498–503.
21. Redmond HP, Schuchter L, Shou J, et al. Interleukin-4 enhances macrophage antimicrobial activity. J Leukoc Biol 1990; 277(suppl 1):97.
22. Johnston RB Jr, Godzick CA, Cohn ZA. Increased superoxide production by immunologically activated and chemically elicited macrophages. J Exp Med 1978; 148:115–127.
23. Lowry OH, Rosebrough AL, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem 1951; 193: 265–275.
24. Aarden LA, De Groot ER, Schaap OL, Lansdorp PM. Production of hybridoma growth factor by human monocytes. Eur J Immunol 1987; 17:1411–1416.
25. Silva AT, Appelmelk BJ, Buurman P, et al. Monoclonal antibody to endotoxin core protects mice from *Escherichia coli* sepsis by a mechanism independent of tumor necrosis factor and interleukin-6. J Infect Dis 1990; 162:454–459.
26. Murray HW. Interferon-gamma, the activated macrophage, and host defense against microbial challenge. Ann Intern Med 1988; 108: 595–608.
27. Livingston DH, Malangoni MA. Interferon-γ restores immune competence after hemorrhagic shock. J Surg Res 1988; 45:37–43.
28. Hershman MJ, Polk HC, Pietsch JD, et al. Modulation of infection by gamma interferon treatment following trauma. Infect Immun 1988; 56:2412–2416.
29. Galandiuk S, Appel S, Pietsch J, et al. Murine intra-abdominal abscess: Immune modulation with interferon-gamma. 2nd International congress on the immune and metabolic consequences of trauma, shock and sepsis mechanisms and therapeutic approaches. Munich, Germany, March 1991.
30. Heremans H, VanDamme J, Dillen C, et al. Interferon γ, a mediator of lethal lipopolysaccharide-induced Shwartzman-like shock reactions in mice. J Exp Med 1990; 171:1853–1869.
31. Heinzel FP. The role of IFN-γ in the pathology of experimental endotoxemia. J Immunol 1990; 145:2920–2924.
32. Paul PE, Ohara J. B-cell stimulatory factor-1/interleukin-4. Annu Rev Immunol 1987; 5:429–459.
33. Stuart PM, Zlotnik A, Woodward JG. Induction of class I and II MHC antigen expression on murine bone marrow-derived macrophages by IL-4 (B-cell stimulatory factor 1. J Immunol 1988; 140:1542–1547.
34. Crawford RM, Finbloom DS, Ohara J, et al. B cell stimulatory factor-1 (interleukin-4) activates macrophages for increased tumoricidal activity and expression of Ia antigens. J Immunol 1987; 139:135–141.
35. McBride WH, Economou JS, Nayersina R, et al. Influences of interleukins 2 and 4 on tumor necrosis factor production by murine mononuclear phagocytes. Cancer Res 1990; 50:2949–2952.
36. Mayoral JL, Schweich CJ, Dunn DL. Decreased tumor necrosis factor production during the initial stages of infection correlates with survival during murine gram negative sepsis. Arch Surg 1990; 125:24–28.

DISCUSSION

DR. JOHN MANNICK (Boston, Massachusetts): I think the key question is, what is the take-home message for the treatment of seriously injured patients from this study? Unfortunately I am uncertain about that.

The problem, it seems to me, is that the kind of critically injured patient that one has to deal with in the intensive care unit probably has not got the profile of cytokine production that is seen in the model that Dr. Daly has described to you, which is a model produced by a lethal or near-lethal injection of endotoxin as a bolus.

What we generally see in the critically injured patient is a situation in which there is a massive and continual hypersecretion of the pro-inflammatory cytokines interleukin-1 (IL-1), IL-6, and tumor necrosis factor (TNF), presumably by monocytes and macrophages, although possibly also by polymorphonuclear leukocytes. But what one sees on the other side of the equation is poor or no production of interferon gamma (IFN-γ) and IL-2 by T lymphocytes.

So the situation that the critically injured patient is presenting us with does not really resemble this model very much. And I am concerned therefore as to the relevancy of the specific recommendation to try to get rid of IFN-γ if one is talking about the patient that is in our intensive care unit after being burned or after multiple trauma.

I think it is also quite true that removing some cytokines initially after a quick challenge with intravenous bacteria or intravenous endotoxin is quite beneficial. For example anti-TNF antibodies will prevent death under those circumstances in experimental animals.

Unfortunately in animal models more resembling the critically injured human, a burned animal, for example, that is then subjected to an infectious challenge, the outcome is different. If you give TNF antibody to such an animal, all of them die, so I think the application of anti-IFN-γ to critically injured humans remains in doubt.

I do think the general principle outlined by Dr. Daly and his co-workers, however, is a valid one, and that is that what we probably need to do with these individuals is to modulate cytokine secretion, particularly to downregulate TNF secretion, as well as to preserve the function of the helper T cell, and that strategies designed to do this, and do this safely, are what we should all be aiming for.

DR. G. MACHIEDO (Newark, New Jersey): I think that we are seeing an evolution of the study of cytokines in the problems of sepsis and trauma. We have moved from phenomenology through mechanistic studies and now we are moving into therapy. And as we move into therapy, I think it is very important to identify both the exact stage of the disease that we are treating, and to define what that disease really is.

Although bolus and endotoxin is a very effective mechanism for studying certain mechanisms, as Dr. Mannick alluded to, I do not think that it is clinically relevant because we rarely have patients go from a normal baseline immune status to a sudden bolus of endotoxemia.

In the manuscript, John, you mention that interferon gamma might be helpful in immunosuppressive states, but we have to realize that the trauma patient—and there are a lot of data to support this—is actually in an immunosuppressed state early on.

We have also shown from our institution, and the data have been confirmed from the group in Denver, that patients suffering from significant trauma and hypotension have a very early bacteremia after trauma, so that would then lead me to two questions:

First of all do you have any data where the order of administration of your interferon gamma and your LPS was reversed, that is, the therapeutic or actually prophylactic agent was given before the priming of the macrophage with LPS?

And secondly have you used interferon gamma in models that more realistically simulate the clinical substance, such as cecal ligation and puncture? And if so, was the agent given before or after the development of the septic syndrome?

Again I think the work is extremely important. I think it is going to advance our knowledge of cytokines and sepsis.

DR. E. FAIST (Munich, Germany): The key message for me resulting from this investigation is as follows: Gamma interferon and, most likely to a much lesser degree, IL-4, are lymphokines that, in a state of homeostasis, represent an absolute precondition for an intact immune response, especially in terms of the sustenance of adequate monocyte function and its antigen-presenting capacity.

In a state of massive trauma, however, evidently there is a crucial defect of gamma interferon synthesis, thus potentially requiring the substitution of this mediator. As we have heard from the authors, however, in a state of endotoxemia, even small amounts of gamma interferon have a deleterious effect on the host defense system.

These findings exemplify how difficult it is to make decisions in terms of immunomodulatory approaches. We always must ask ourselves: Are we sure to induce immunoenhancement with a certain intervention or is there the risk to trigger an immunosuppressive pathway? This question can only be answered *in vivo* when we sufficiently inform ourselves about the state of monocyte activation. So far today under a nonexperimental but under a clinical situation, it is not yet possible with the currently available methods of immunoscreening to decide safely at the bed side.

This study in general, I think, stresses the role of the monocyte as the crucial cell for the septic response, and the necessity to elucidate all aspects of behavior patterns of this cell.

I have one question for Dr. Daly and his co-workers. Did you in any part of your experiments look for the expression of the Ia antigen as a marker for facilitatory monocyte function?

I do thank the Association for the privilege of discussing this paper.

DR. D. MORTON (Los Angeles, California): My reason in rising is to support his observations in humans, in one clinical situation in patients who are receiving IL-2 for immunotherapy.

We have measured various cytokines in serum, and the one cytokine that correlated best in this model in man was gamma interferon.

At least in this particular situation, the neutralization of gamma interferon may in fact ameliorate some of the toxic effects of IL-2, and I think that that certainly is relevant to your model.

DR. H. PAUL REDMOND (Closing discussion): In reference to Dr. Mannick's questions. Dr. Mannick referred to trauma patients and septic patients and seemed to blend them together. Our model really referred to the septic patient and not to the traumatized patient, and it is very important to identify the difference between these two groups.

In the situation of trauma, we have a situation of immunosuppression, and indeed in a situation such as that, activating agents such as interferon gamma (IFN-γ) and interleukin-4 appear to be beneficial. I think Dr. Polk's work has shown that where he has administered subcutaneous IFN-γ to traumatized animals and noted that he can upregulate monocyte class 2 expression, and indeed enhance survival.

Our model, however, is a model of septic shock, and really in this situation the monocyte or macrophage is totally opposite to what happens in trauma. It is activated. In other words you get enhanced free radical production, enhanced cytokine production, enhanced class 2 expression, and perhaps enhanced antigen presentation.

Sepsis and trauma can occur together, but we are trying to decipher what is going on in septic situations. In a situation where you have pure sepsis, then a quelling down or an immunomodulation of this hyperimmune responsiveness that occurs early in the septic stage may be beneficial.

Secondly in reference to Dr. Machiedo's questions, he mentioned that our model of LPS injection may not be relevant. Well I am glad to say, Dr. Machiedo, that we have gone on from that, and we have looked at a cecal ligation and puncture model, and also an *Escherichia coli* infusion model.

First in relation to *E. coli*, once again, monoclonal antibodies against IFN-γ, as you would expect from the endotoxin studies, were significantly more beneficial than either IgG-1 isotope control antibody or IFN-γ. Let me state that IFN-γ was not as detrimental as it was when simultaneously administered with endotoxin. It did not appear to have the same synergy.

In terms of cecal ligation and puncture, administration of monoclonal antibodies to IFN-γ 2 hours after cecal ligation and puncture was significantly beneficial in prolonging median survival, as measured by log rank analysis using both Wilcoxon tests and other forms of statistical analysis.

Very interestingly, the monoclonal antibody to IFN-γ was only beneficial when we gave it 2 hours afterwards. I think getting back to Dr. Mannick's questions, if you give the monoclonal antibody in divided doses over time after cecal ligation and puncture, the animals start to die much more rapidly, suggesting that in the initial period of septic shock that develops after cecal ligation and puncture, the monoclonal antibody modulates the host in a beneficial way, but that as you develop, say, the abscess, or as you develop the chronic septic insult after cecal ligation and puncture, then administration of that monoclonal antibody may be bad for you because your immune system is depressed.

Interestingly in the cecal ligation and puncture studies, administration of anti–IL-4 was beneficial.

In other words to summarize the two cecal ligation and puncture studies: if you give monoclonal antibody to IFN-γ as one dose, it is significantly beneficial. If you give monoclonal antibody IL-4 in one dose, it is not beneficial, but it was beneficial in divided doses, and that sort of leaves us in a quandary as to where to go next with our studies.

Dr. Faist asked a question about class 2 antigen expression. All I can say is, Dr. Faist, we have looked at that *in vitro*, and if you use endotoxin and IFN-γ *in vitro*, they will significantly upregulate class 2 expression. If you look at the functional representation of class 2 expression, that being antigen presentation, and if you do it in the presence of IFN-γ, high levels of it, it downregulates antigen presentation. So I am beginning to think that perhaps class 2 does not accurately reflect functional antigen presentation.

Analyses of Preventable Deaths by Mechanism of Injury Among 13,500 Trauma Admissions

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Preventable deaths (PD) were evaluated by mechanism of injury for 13,500 trauma admissions to eight hospitals over 2 years. There were 42 (3.3%) hospital deaths. Preventable deaths were analyzed by time of death, anatomic site of injury, and mechanism; penetrating (PEN) and blunt with low fall (LF) injuries were considered separately. Preventability of death for patients with probability of survival of less than 0.5, "unexpected deaths," after penetrating and blunt injuries, was determined by consensus of three trauma surgeons. Twelve per cent of deaths were found to be possibly preventable. The incidence of preventable deaths did not differ significantly across groups. Factors in preventable deaths varied by injury cause: delays in operation, PEN (50%), and blunt injury patients (48%); management errors, blunt (52%) and LF (84%); and technical errors, PEN (37.5%). Median times to death were significantly different by cause of injury: PEN, 3 hours; blunt, 13 hours; and LF, 3975 hours. Problems were identified in the hospital care of patients, especially those with LF, leading to sepsis and multiple organ failure.

IN GENERAL, PREVENTABLE death studies have been performed to assess factors that, if remedied, could result in increased survival rates among injured patients. In 1985, Cales and Trunkey[1] reviewed 29 preventable death studies and found that they fell into two general groups: those that evaluated care where a trauma system did not exist, and those that assessed the impact of a systematized approach to trauma care. Of the 29 studies reviewed, 20 focused entirely on motor vehicle crash patients, whereas several focused only on abdominal injuries.[2,3] Since 1979, there have been only two studies that included trauma of most causes; both were from San Diego County.[4,5] The present study included hospitalized adult deaths from all causes except burns.

From the Institute for Trauma and Emergency Care, New York Medical College, Valhalla, New York

There have been many variations in the designs of preventable death studies. Some used stratified samples,[6] others used complete samples[7]; some used explicit criteria[8] and others implicit criteria.[9]

In this study the American College of Surgeons Committee on Trauma TRISS method was used to identify patients whose deaths were considered to be unexpected.[10] A panel of trauma surgeons then evaluated these unexpected deaths to determine which were possibly preventable.

Methods

Setting and Selection of Cases

The study was conducted by the Institute for Trauma and Emergency Care (ITEC) at the New York Medical College (NYMC). Eight hospitals, all affiliates of NYMC, participated in the research. Hospitals ranged in size from 200 to 813 beds. Three of the eight hospitals were officially designated level I trauma centers. Average numbers of trauma admissions per month ranged from 32 to 248, whereas average numbers of adult major trauma admissions eligible for the study ranged from 12 to 75 per month.

Logs of all emergency department admissions in each hospital were reviewed by one of three trained nurse-abstractors. Patients were included if they either died or stayed in the hospital for 48 hours or more. Patients with major burns or only distal fracture (fractures below the knee or elbow) were excluded.

Data Collected

A study record was opened for each patient on his or her identification as eligible. Information from prehospital

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and emergency department records was abstracted at that time and was entered on standardized forms. The complete hospital record was reviewed after the patient's discharge, and final information regarding the patient's stay and condition at discharge were recorded.

A wide range of data elements were collected regarding the care of each patient. For the purpose of this paper, important elements included demographic data, information regarding the cause of injury, and trauma diagnoses required to calculate the Abbreviated Injury Score (AIS),[11] Injury Severity Score (ISS),[11] Revised Trauma Score (RTS),[12] and the probability of survival (Ps) using the TRISS method, ISS, RTS, and age.[10] Data concerning comorbidity and complications also were collected. Assessments of the preventability of death were made by reviewing the entire study abstract.

The Abbreviated Injury Score (AIS) assesses injury severity on a scale of 1 (minor) to 6 (fatal) in each of several body regions.[11] Scores are subjective assessments of severity made by expert raters. Ratings are completed on the basis of reviewing emergency department and hospital records. Each of six regions is scored, with the highest AIS value given to any injury in that area. Abbreviated Injury Score values for the three highest scoring body regions then are squared and summed to form the ISS.

In this study AIS codes were assigned on the basis of the assessments of trained nurse-raters. American Association of Automative Medicine (AAAM)-1985 coding guidelines were followed.[11] These generally parallel the guidelines used by the American College of Surgeon Major Trauma Outcome Study (MTOS) guidelines for 1988.[13] Three differences between MTOS coding and ours deserve description, however. Under MTOS, initial loss of consciousness is not scored, named vessel lacerations (not further specified) are coded as "major," and hemomediastinum is not coded but is used to upgrade. Using AAAM guidelines, we scored initial loss of consciousness, gave a lower code to named vessel lacerations (not further specified), and coded hemomediastinum. To our knowledge there have been no studies of the effect of such differences in guidelines on AIS scores; however it seems unlikely that the effect is great.

The RTS includes three physiologic parameters (Glasgow Coma Scale [GCS] systolic blood pressure, and respiratory rate) recorded soon after the patient's injury, generally in a prehospital or emergency department setting.[12] The coefficients used in the formula were developed using regression analyses based on data for 2000 Washington Hospital Center patients and tested using information concerning the more than 25,000 patients in the MTOS.[10]

The TRISS methodology, also developed by Champion et al.,[12,14] combines the ISS, the RTS, and age to produce a Ps score.[10] Regression coefficients were derived from data concerning the 26,000 patients in the American College of Surgeons Major Trauma Outcome Study (MTOS). The formula is $Ps = 1/(1 + e - b)$, where $b = b0 + b1 (RTS) + b2 (ISS) + b3 (age)$. Age is dichotomized as 0 for patients under 55 years and 1 for patients 55 or older. Separate sets of regression coefficients were developed for blunt and for penetrating injury groups. A *z* score is used to determine whether differences between the number of observed deaths within any given group of patients is significantly different from the number predicted. A negative *z* score indicates an excess of observed over predicted deaths. An M score accompanies each *z* score and indicates whether there is a reasonable match with respect to the mix of injury severity between the test data set and the MTOS set; an M score of at least 0.88 is considered desirable.

Analyses

Analyses proceeded in five stages. First patients for whom full data required to compute Ps were available were compared with those for whom information was missing, using chi square and t test to evaluate the significance of differences.

Second the reliability of AIS coding among raters is critical to the generalizability of results. Following the method developed by MacKenzie et al.,[15] kappa and weighted kappa were used to evaluate levels of agreement with regard to AIS coding, and the intraclass correlation coefficient was used to assess the reliability of ISS coding.

In the last two sets of analyses, patients were classified by cause of injury: penetrating (including gunshot, stab, and bite wounds), blunt (including motor vehicle crashes, falls from heights, and assaults with blunt objects), and low falls (on level surface or from low steps identified by E-codes 880.9, 884.2, 885.0, 888.0). (Although other studies have analyzed motor vehicle crash patients separately, we found no significant differences in death, unexpected death rates, or preventability between these patients and those falling from heights or assaulted with blunt objects; thus the categories were combined.) Comparisons among groups with respect to selected demographic and injury characteristics were made using t tests, analyses of variance, chi square tests, and *z* scores.

"Unexpected deaths" were defined as patients who died of any injury cause with a Ps of 0.5 or greater. A panel of three experienced general surgeons (CGC, WMS, NA) reviewed a detailed abstract for each unexpected death and independently evaluated whether the death was "possibly preventable" (hereafter "preventable"). Three general surgeons were chosen for the review panel based on the recommendation of Lowe et al., who reported difficulties with an interdisciplinary panel.[8] The panel of surgeons then met, reviewed each death, and arrived at a consensus for each case. All unexpected deaths were reviewed for penetrating and blunt injury patients. Among low falls patients, previous research[16] has suggested a strong association between three comorbidities—anemia, chronic obstructive pulmonary disease (COPD), and congestive heart failure (CHF)—and death. Thus low falls patients with unexpected deaths and any of these three comorbidities were unlikely to be deemed unpreventable and were not reviewed. Abstracts for low falls patients with unexpected deaths and neither COPD, CHF, nor anemia were reviewed for preventability. Unexpected deaths and preventable deaths then were compared in terms of cause of injury, cause of death, and survival patterns.

Results

The study included 13,500 adult patients admitted from July 1, 1987 through June 30, 1989. As Figure 1 shows, 5686 (42.1%) met the eligibility criteria for abstraction. Most of those not included had distal fractures or dislocations, or were discharged in less than 48 hours. Some element or elements of data required to calculate the first emergency department RTS portion of the Ps were missing for 68 (1.2%) of the 5686 cases. Most of these cases were interhospital transfers for whom one or more value needed from the first hospital emergency department were not included in the medical record of the receiving hospital. In general these patients were injured in motor vehicle crashes (52.9%), and were somewhat more severely injured than the patients analyzed, as evidenced by their higher mean ISS values (16.7 *vs.* 12.2). There was no significant difference in death rates between those with *versus* those without Ps values. Of the 5618 patients with Ps values, 421 (7.5%) died, and four (5.9%) of those without Ps values did not survive to hospital discharge.

Interrater agreement between pairs of nurse-raters with regard to AIS and ISS coding was tested for 72 cases. For AIS coding, kappas ranged from 0.64 to 0.74; weighted kappas ranged from 0.68 to 0.79. The intraclass correlation coefficient used to evaluate the reliability of ISS coding was 0.78. These results are comparable to those reported by MacKenzie et al.,[15] and suggest very good agreement among raters.

In the overall sample, 20.5% (1151) of patients sustained penetrating injuries, whereas 34% (1913) had low falls, and nearly 46% (2554) suffered other blunt injuries. Among the 421 patients who died, and who constituted the effective sample for this study of preventable deaths, 28% had penetrating injuries, 30% low falls, and 42% other blunt injuries (Fig. 2). Mortality rates ranged from 6.7% among low falls patients and 6.9% among blunt injury patients to 10.2% among those having penetrating injuries. On average penetrating injury patients who died were the youngest (31.8 ± 11.4 years), whereas low falls patients who died were the oldest (82.7 ± 6.8 years) (Table 1). Average ISS values were highest among those dying of blunt injuries (37.2 ± 15.4) and lowest for the low falls groups (11.8 ± 6.8). The mean RTS was lowest for the penetrating injury group (3.1 ± 2.8) and highest among those dying from low falls (7.4 ± 1.3). Mean Ps values calculated using the TRISS method were 0.33 for those dying of penetrating injuries, 0.43 among blunt injured patients, and 0.91 for those having low falls. Significant

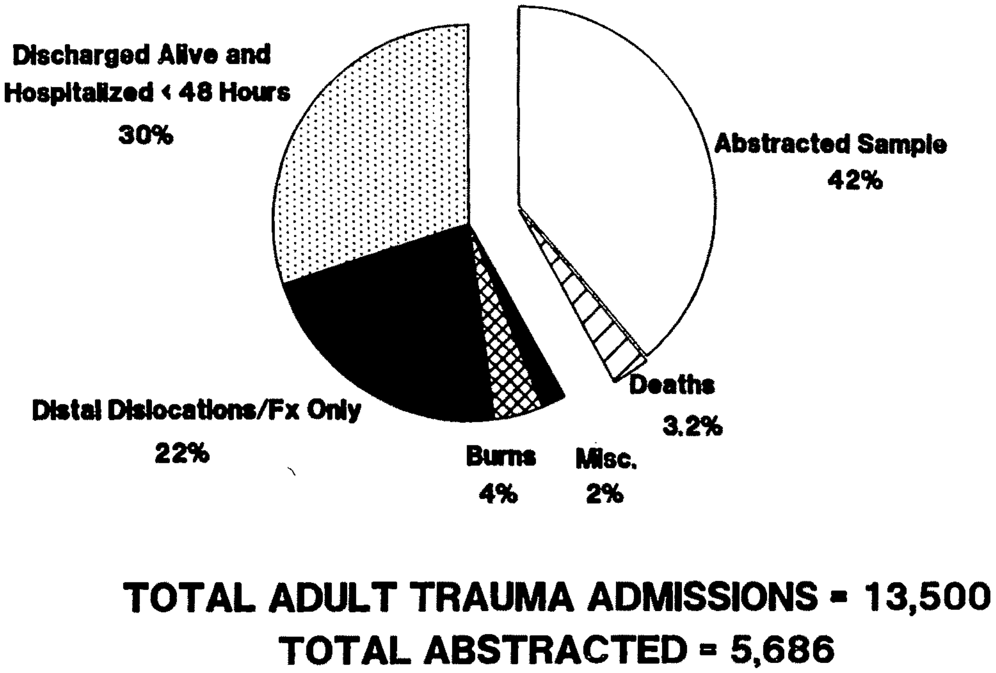

FIG. 1. Adult trauma admissions (July 1, 1987–June 30, 1989).

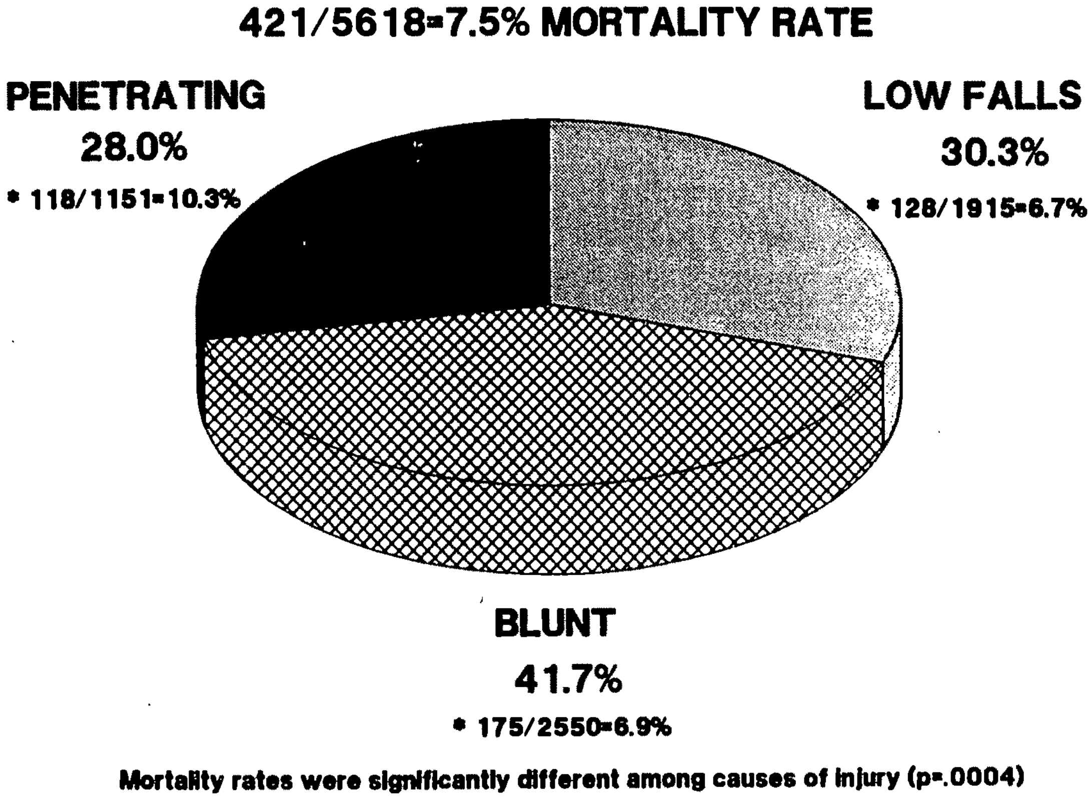

FIG. 2. Cause of injury.

and negative z scores for the penetrating injury and low falls groups indicated that there were more deaths than expected on the basis of the MTOS in those groups. The z score for the blunt injury group was not significant.

Figure 3 shows proportions of deaths that were unexpected ($Ps \geq 0.5$) and that were deemed preventable by the panel of surgeons. Nearly 55.3% of deaths overall were unexpected, whereas 11.9% were deemed preventable by the physician panel. Unexpected death rates ranged from 28.8% among penetrating injury patients to 45.7% among blunt injury patients and 93% among those with low falls ($Pr < 0.05$) (Fig. 4). The preventable death rates were 6.8% for those with penetrating injuries and 13.1% for those with blunt injuries. The abstracts of 69 (53.9%) low falls patients without COPD, CHF, or anemia were reviewed for preventability. The deaths of 19 (14.8% of all low falls patients) were held to be preventable. Differences in rates of preventable deaths were not statistically significant across groups.

Most unexpected deaths among penetrating and blunt injured patients were related to hemorrhage and brain injury (Fig. 5). Sixteen of thirty-four unexpected penetrating injury deaths were related to hemorrhage; four (25%) were deemed preventable. Three of seven (42.9%) deaths among penetrating injury patients with sepsis/multiple organ failure were considered preventable. Among blunt injury patients, over half of the unexpected deaths were related to CNS; ten (22.7%) were deemed preventable. Eight of 17 (47.0%) deaths among blunt injury patients with sepsis/multiple organ failure were held to be preventable. Approximately half of the unexpected deaths among low falls patients were related to cardiovascular diseases; 11 (31%) were deemed preventable.

The high unexpected death rate (93%) among low falls patients relates to the singular nature of this group. Over half suffered hip fractures and an additional 13.5% had neck, trunk, or other relatively minor skeletal injuries (Fig. 6). These are injuries that produce relatively low ISS val-

TABLE 1. *Characteristics of Patients Who Died*

Variable	PEN (118)	Blunt (175)	Low Falls (128)
Age (mean + SD)	31.8 ± 11.4	43.4 ± 19.6*	82.7 ± 11.9†
ISS (mean + SD)	30.3 ± 12.0	37.2 ± 15.4*	11.8 ± 6.8†
RTS (mean + SD)	3.1 ± 2.8	3.9 ± 2.8*	7.4 ± 1.3†
Ps (mean + SD)	0.33 ± 0.36	0.43 ± 0.38*	0.91 ± 0.20†
Z-score	−2.64	−1.63	−9.34
M-score	0.95	0.98	0.88

* $p < 0.05$ PEN *versus* blunt.

† $p < 0.05$ LF *versus* PEN and blunt.

PEN, penetrating injury; ISS, Injury Severity Score; RTS, Revised Trauma Score; Ps, probability of survival.

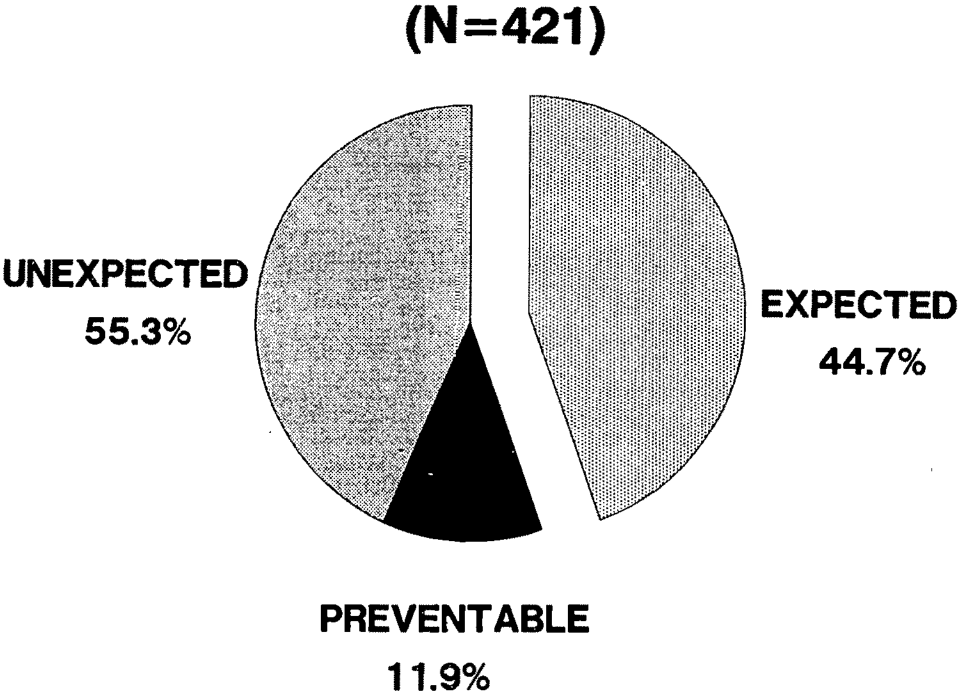

FIG. 3. Classification of deaths.

ues, high RTS values, and thus high Ps values. The comorbidity and complication rates within this group, however, were high. Low falls patients were significantly more likely than either penetrating or blunt injury patients to have CHF, COPD, anemia, hypertension, or renal failure (Fig. 7), whereas mean numbers of complications were 3.0, 0.6, and 1.4, respectively (Pr < 0.01). This combination of high Ps values and high rates of complications and comorbidities largely explains the high unexpected death rate.

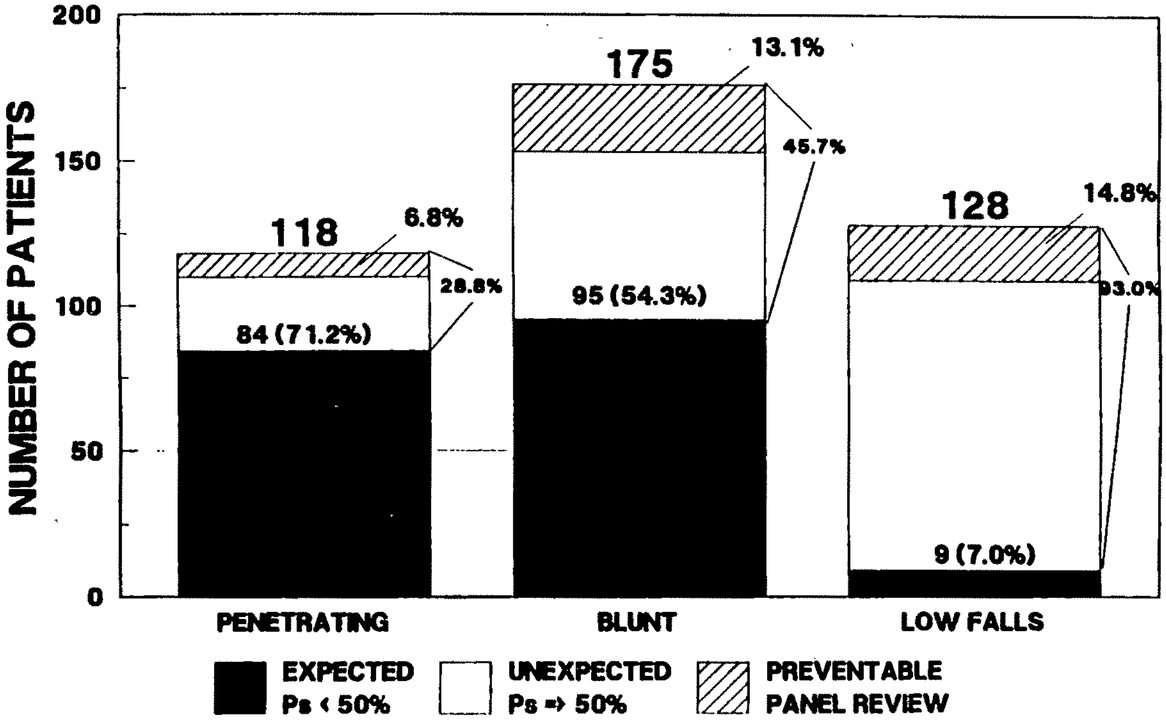

FIG. 4. Classification of deaths by cause of injury.

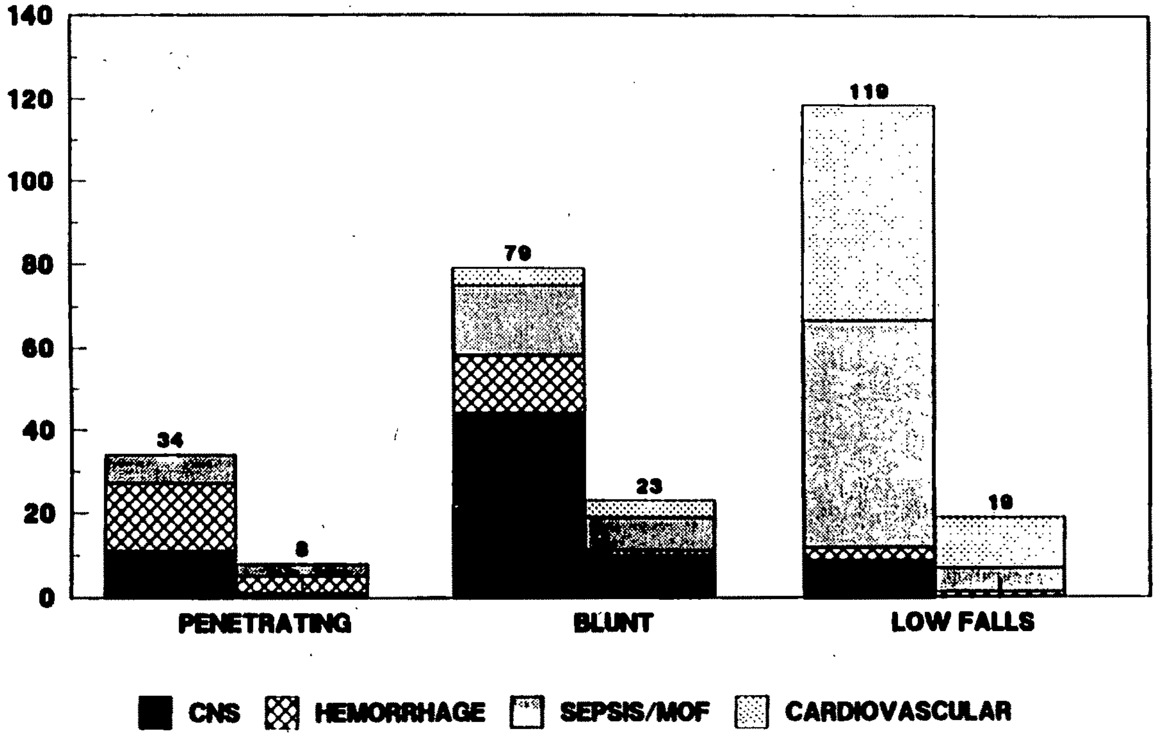

FIG. 5. Unexpected and preventable deaths by cause by death.

Three broad classes of reasons for preventable deaths were identified (Fig. 8): delays in operation, management errors, and technical errors. Most (58%) preventable deaths resulted from management errors; an additional 34% were caused by delays in operations, and 8% by technical errors. Delays in operations occurred most frequently among patients with head injuries, and, secondarily among patients with penetrating chest injuries. Errors in management occurred primarily among those with low falls or other blunt injuries who had prolonged hospital stays. The errors involved the inability to prevent and effectively treat complications such as pneumonia, pulmonary embolus, and organ system failure in patients who should have recovered from their injuries. Eleven of these developed pulmonary emboli, whereas eight bedridden patients survived their injuries but developed pneumonia, sepsis, respiratory failure, or acute renal failure. Technical errors occurred primarily among pene-

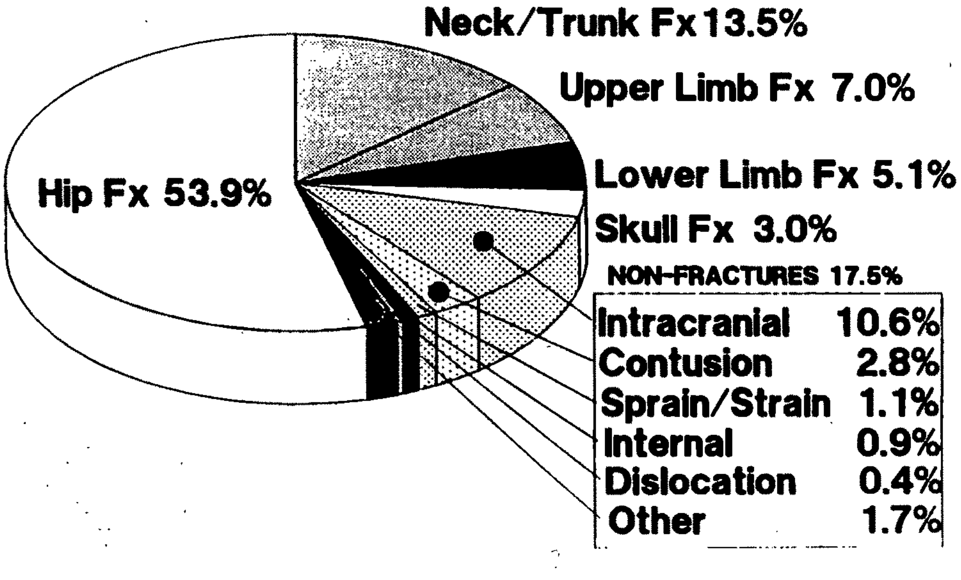

FIG. 6. Primary diagnosis for low-fall patients (N = 1931).

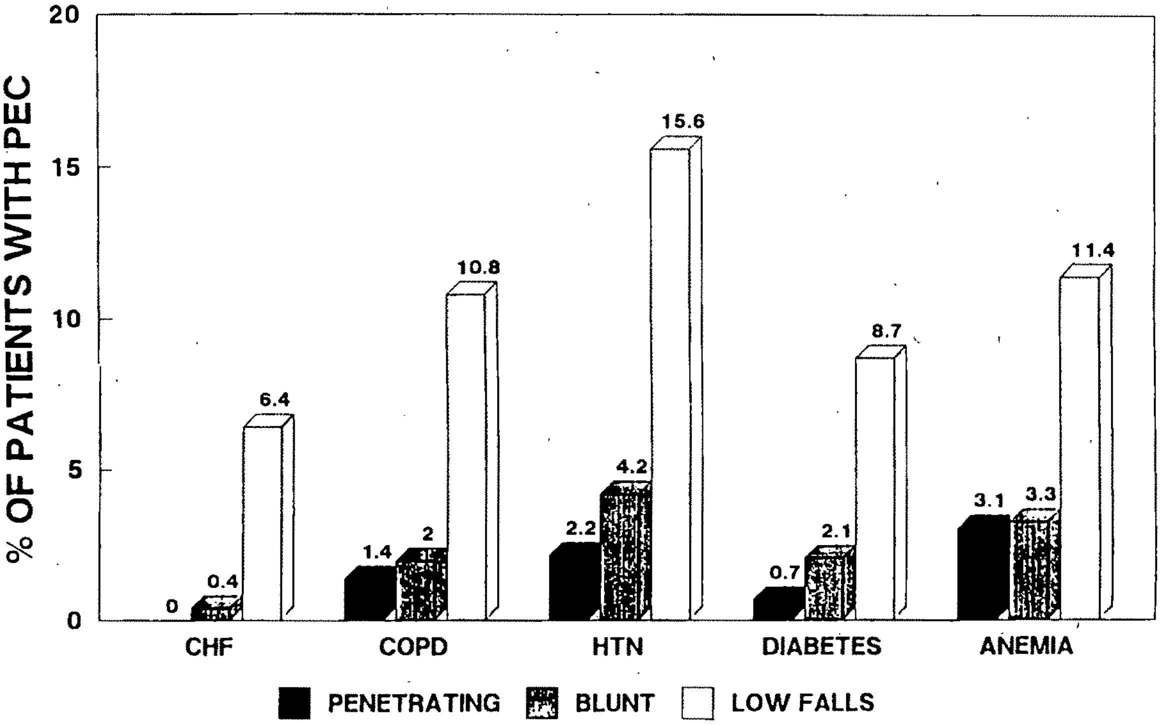

FIG. 7. Prevalence of pre-existing conditions by cause of injury.

trating injury patients and included failure to control femoral vein transection, delayed hemorrhage from a tracheostomy site, failure to recognize and control a hepatic artery injury, and an innominate vein injury secondary to catheter insertion.

Deaths are classified with respect to injury cause and time to death in Figure 9. Mean times to death were significantly different among groups (Pr < 0.05). Penetrating injury patients who died survived an average of 55 hours (median, 3 hours). Over three quarters died within 24

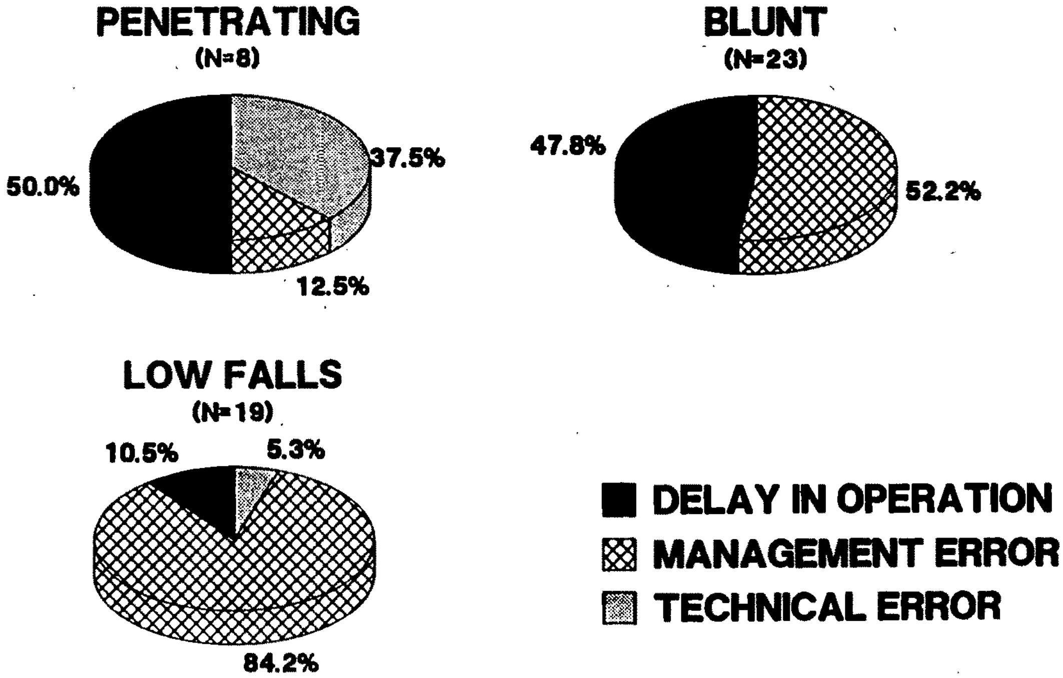

FIG. 8. Reasons for preventable deaths.

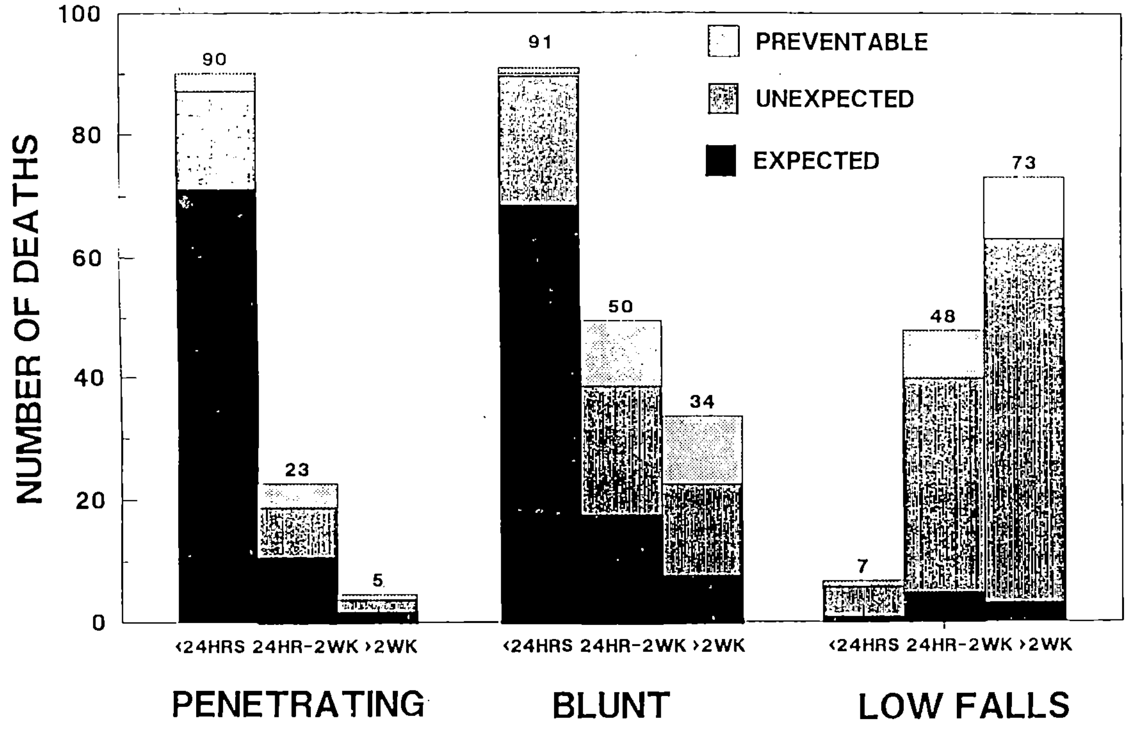

FIG. 9. Classification of deaths by cause and time to death.

hours, whereas just 4.2% died after 2 weeks. Early deaths tended to be due to exsanguination or gunshot wounds to the head. Fifty-six per cent of unexpected and three of eight preventable penetrating injury deaths occurred within the first 24 hours of hospitalization. Among patients dying of blunt injuries, the median hour of death was 12.75, with 53% dying within 1 day and nearly 20% dying after being hospitalized for more than 2 weeks. Forty per cent of unexpected blunt injury deaths occurred between 2 and 14 days after hospitalization; just one preventable death occurred within the first 24 hours of hospitalization. Low falls patients had the longest mean and median times to death (634 and 396.5 hours, respectively). Fifty-seven per cent of these patients died more than 2 weeks after entering the hospital. Nearly 60% of unexpected deaths and 53% of preventable deaths occurred after 2 weeks of hospitalization. Deaths within this group were related primarily to sepsis/multiple organ system failure and to cardiovascular disease.

Discussion

Preventable death studies have been done in many ways by different investigators throughout the country.[1] Sample selection methods have varied widely: many studies focused primarily on motor vehicle crash patients[2,6,17,18] whereas others included only pediatric injuries.[4] In some instances deaths occurring in a short period in a given area have been studied,[19] whereas in others, longitudinal stratified samples have been drawn.[6] Some studies have focused on hospital deaths,[20] and others have included prehospital deaths.[2] The sizes of panels deciding on preventability have varied (from 3 to 35) and, although most have included only surgeons, the mix of specialties represented has varied.

Definitions of "preventability" also have differed. Frames of reference for preventability have varied from optimal care, to the level of care "generally expected" in the community, to the level available "if the injury occurred in the accident department of a general hospital." Criteria for preventability ranged from completely implicit to explicit. Data sources have varied from the use of all hospital and prehospital records, to the inclusion of only hospital or only autopsy records.[21] The definition of consensus among panel members has differed; one study required agreement between two of six panel members,[8] whereas most required a simple majority.[1]

The wide disparity among methods has resulted in a lack of comparability among results. If preventable death studies were to be standardized with regard to several of these parameters, their usefulness as an evaluative tool within and among sites would be enhanced greatly.

Several years ago the American College of Surgeons Committee on Trauma (ACS/COT) adopted a quality assurance protocol based on the MTOS.[22] The protocol suggested that the TRISS method be used to identify patients whose combinations of injury cause, anatomic and physiologic status, and age yielded a Ps of 0.5 or greater but who died, *viz.*, who had at least a 50% chance of living but who died. These cases then would be considered "unexpected deaths." It was further recommended that detailed peer review of such deaths be conducted because it is within this group that deficiencies in individual patient care and in trauma systems could be most readily identified.[22]

The research reported is the first to tie the ACS/COT quality assurance recommendations to a preventable death study. In it the deaths of patients whose Ps was less than 0.5 were considered to be expected whereas those who died with a Ps of 0.5 or greater were deemed unexpected. Thus an objective severity scale was used as an audit filter. Preventability then was assessed on a case-by-case basis, with one exception—unexpected deaths among low falls patients with either COPD, CHF, or anemia were considered to be unpreventable and not reviewed.

The results of the study confirm and expand those reported by others. Shackford et al[23] reported on 224 nontrauma hospital deaths and 541 trauma center deaths. At the nontrauma center hospital, 7.6% were considered frankly preventable, whereas at the trauma center there were 2.0% such patients. Among the explicit guidelines used were: nonpreventable patients should have $Ps < 0.25$, potentially salvageable $0.50 > Ps > 0.25$, and frankly preventable $Ps > 0.50$. Thus, Shackford and co-workers' frankly preventable group corresponds to our possibly preventable group. They found that preventable deaths occurred at nontrauma hospital because the severity or multiplicity of injuries was not appreciated. Preventable deaths at trauma centers were due to an error in either judgment or technique. We did not attempt to compare trauma center with nontrauma because three of our five nontrauma centers were comparable in capabilities to level II trauma centers, which are considered trauma centers in the Shackford study. The death of only 1 of 12 (8.3%) penetrating injury patients, who died of central nervous system (CNS) damage was found to be preventable; this result is consistent with Gennarelli and co-workers'[13] report that the most lethal wound is a gunshot wound to the head, yielding a mortality rate of 68%.

Others[2,3,17,18,24,25] have reported 25% to 53% preventable death rates among patients in motor vehicle crashes. In the present research, the motor vehicle and other blunt injury patients were very similar and were analyzed as one group. The preventable death rate was just over 13%. Over half (44 of 79) of the unexpected deaths were related to CNS injuries. The fact that just 10 were held to be preventable relates in part to deficiencies in the ISS. For example patients with isolated severe head injuries have an AIS of 5 and an ISS of 25. Even with a low Glasgow Coma Score, such a patient is likely to have a Ps of greater than 0.5 and thus be an "unexpected" death. Delays in operations and poor management were the major causes of preventable deaths in this group.

One group of patients notably absent among these blunt injury deaths were patients dying from ruptured spleens. In several studies from the 1960s and 1970s, delays in diagnoses and operations on ruptured spleens accounted for a significant proportion of preventable deaths.[2,3,17,26] In addition many of the earlier preventable death studies of motor vehicle crash victims demonstrated significant deficiencies in recognizing and appropriately managing patients in hypovolemic shock. This study shows that preventability is shifting to longer-term management issues of these patients.

Low falls patients generally have been excluded from preventable death studies because they often are considered not to need the resources of a trauma center. Fitts et al.[7] performed an autopsy study of all trauma deaths in Philadelphia in 1969. They found that 26% of deaths were associated with hip fractures; 30% of patients in the present study died of low falls. These results support Fitts and co-workers' overall conclusion that the most common cause of death from injury was a fall by an aged patients. Fitts et al. also reported that 15% of all patients studied died from pulmonary emboli. We found 47% of our preventable low falls deaths were from pulmonary emboli.

The low falls patients mortality rate is also profoundly effected by age and comorbidity. Morris et al.[27,28] found that, starting at age 40 years, death was independently and adversely influenced by male gender and the presence of both pre-existing medical disease or injury to head or abdomen. Morris et al. found that congenital coagulopathy, cirrhosis, ischemic heart disease, and COPD and diabetes increase the mortality rate in all patients. We found that in hip fracture patients, congestive heart failure, COPD, and anemia increased mortality rate.

The duration of survival of patients with various injury causes was found to be consistent with general experience. Thus penetrating injury patients tended to die early, 76% in less than 24 hours, or to be discharged alive. Approximately half of blunt injury deaths occurred within the first 24 hours, but nearly 20% were late deaths. Shackford et al.,[5] in a study population that was close to 70% blunt trauma, found that 75% died in less than 24 hours. Low falls patients rarely died acutely of their rather minor injuries, but instead died after 2 weeks largely because of complications or comorbidities.

The study has several strengths and limitations. Patients from eight hospitals (3 trauma centers and 5 nontrauma centers) with any injury cause were included. The hospitals were chosen for convenience, however, and, because the study was not population based, the results do not necessarily represent a broader population. All injured patients—except those with burns or only distal fractures—

who died or who stayed in the hospital for at least 48 hours over a 2-year period were included. All medical records—prehospital, hospital, and autopsy records—were used to abstract data. The study also is the only preventable death analysis to document the reliability of the data abstracting process. Prehospital and autopsy data, however, were not available in great detail; such information could have provided a more objective basis for some decisions relative to preventability—particularly with respect to care in the field. In addition we found, as others have suggested, that the longer the patient lived the more difficult it was to determine the preventability of death.[2] Our review panel concluded that, particularly among low falls patients, review of the entire medical record would have provided a better basis for deciding preventability than did review of the study abstract. In particular, a full record review would have provided more opportunity to assess the impact of preexisting medical conditions—conditions most other preventable death studies have neglected.[26] Finally panel members included surgeons who cared for some of the patients reviewed, and implicit criteria based on a presumed community standards of care were used. The study would have been stronger if an independent group of surgeons had performed this study, using explicit criteria.

It could be argued that because preventable death studies are so subjective, perhaps we should simply replace them with the more objective TRISS method. The present TRISS method, however, has certain limitations that preclude its use in this way. First the proportion of unexpected deaths found to be preventable ranged from 17% among low falls patients to 29% among blunt injury patients (22% overall). Thus the sensitivity of the statistic is too low and the false-positive rate too high for it to be used alone to identify preventable deaths. The false-negative rate is unknown—but presumably is not 0—because preventability was not assessed among expected deaths in the present research. The method also does not account for unexpected saves—those surviving with Ps under 0.5. In the present study, unexpected survivors included 17 patients with penetrating injuries, 21 with blunt injuries, and 5 with low falls.

Limitations summarized by Cayten et al.[16] also apply to the use of TRISS in identifying preventable deaths. Thus the broad categories of blunt and penetrating used in TRISS calculations result in wide variation in mortality rates within the major subdivisions of each—particularly with regard to differences between patients with gunshot *versus* stab wounds. The mix of patients in any given study will affect the ratio of expected to unexpected deaths. In addition the RTS has been shown to have virtually no predictive value with respect to the survival of patients with low falls, and the ISS has been found to be quite limited in this regard as well. The facts that 93% of low falls deaths were unexpected in this study and that it was necessary to screen this group for comorbidities supports the suggestion that these patients should be excluded from analyses using the TRISS method. Finally the method is limited in predicting the outcomes of patients with multiple severe injuries to a single body part. For example patients who die of multiple gunshot wounds to the abdomen may well be found to be unexpected deaths using the TRISS method even though the number and severity of their injuries were lethal.

Thus the quantitative TRISS method alone cannot measure whether a death actually is preventable. The recently proposed A Severity Characterization of Trauma (ASCOT) method seems promising but has not been independently tested.[14] But using the Ps as audit filter strengthens the conclusions of preventable death studies because it is more difficult for critics to argue that patients in the unexpected death category had such severe injuries that death was inevitable.

Conclusions

Analyses of unexpected deaths using the TRISS methodology in an eight-hospital study including trauma and nontrauma centers showed 12% of deaths to be possibly preventable. The incidence of unexpected deaths varied significantly according to mechanism of injury; the incidence of preventable deaths did not differ significantly across groups.

Factors in preventable deaths varied by injury cause: delays in operation—most frequent among penetrating and blunt injury patients; management errors—most frequent among blunt and low falls patients; technical errors—most frequent among those with penetrating injuries.

Compared with previous studies, there were fewer failures of diagnosis and treatment of abdominal trauma. There remained a problem with early diagnosis and treatment of severe head injury. Problems were identified in the long-term care of post-trauma patients, especially low falls patients, leading to sepsis and multiple organ failure. The advantages and disadvantages of the use of TRISS as an audit filter for preventable deaths are described.

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References

1. Cales RH, Trunkey DD. Preventable deaths: a review of trauma care systems development. JAMA 1985; 254(8):1059–1063.
2. Foley RW, Harris LS, Pilcher DB. Abdominal injuries in automobile accidents: review of care of fatally injured patients. J Trauma 1977; 17:611–615.
3. Gertner HR, Baker SP, Rutherford RB, Spitz WU. Evaluation of the management of vehicular fatalities secondary to abdominal injury. J Trauma 1972; 12:425–431.
4. Neuman TS, Bockman MA, Moody P, et al. An autopsy study of traumatic deaths: San Diego County, 1979. Am J Surg 1982; 144:722–727.
5. Shackford SR, MacKersie RC, Davis JW, et al. Epidemiology and pathology of traumatic deaths occurring at a level i trauma center in a regionalized system: the importance of secondary brain injury. J Trauma 1989; 29:1392–1397.
6. Cales RH. Trauma mortality in Orange County: the effect of implementation of a regional trauma system. Ann Emerg Med 1984; 13:1:1–9.
7. Fitts WT, Lehr HB, Bitner RL, Spelman JW. An analysis of 950 fatal injuries. Surgery 1964; 56:663–668.
8. Lowe DK, Gately HL, Goss JR, et al. Patterns of death, complication, and error in the management of motor vehicle accident victims: implications for a regional system of trauma care. J Trauma 1983; 23:503–509.
9. West JG, Trunkey DD, Lim RC. Systems of trauma care—a study of two counties. Arch Surg 1979; 114:455–459.
10. Boyd CR, Tolson MA, Copes WS. Evaluating trauma care: the TRISS method. J Trauma 1987; 27:370–378.
11. American Association of Automotive Medicine. The Abbreviated Injury Scale. Arlington Heights, IL, 1985.
12. Champion HR, Sacco WJ, Copes WS, et al. A revision of the trauma score. J Trauma 1989; 29:623–629.
13. Gennarelli TA, Champion HR, Sacco WJ, et al. Mortality of patients with head injury and extracranial injury treated in trauma centers: J Trauma 1989; 29:1193–1202.
14. Champion HR, Copes WS, Sacco WJ, et al. A new characterization of injury severity. J Trauma 1990; 30(5):539–546.
15. MacKenzie EJ, Shapiro S, Eastham JN. The abbreviated injury scale and injury severity score. Medical Care 1985; 23:6.
16. Cayten CG, Stahl WM, Murphy JG, et al. Limitations of the TRISS method for interhospital comparisons: a multihospital study. J Trauma April 1991; 31:471–482.
17. Certo TF, Robers FB, Pilcher DB. Review of care of fatally injured patients in a rural state: 5-year followup. J Trauma 1983; 23: 559–565.
18. Kreis D, Plascencia G, Augenstein D, et al. Preventable trauma deaths: Dade County, Florida. J Trauma 1986; 26:649–654.
19. Campbell S, Watkins G, Kreis D. Preventable deaths in a self-designated trauma system. The American Surgeon 1989; 478–480.
20. Moylan JA, Detmer DE, Rose J, Schulz R. Evaluation of the quality of hospital care for major trauma. J Trauma 1976; 16:517–523.
21. West JG, Yurko AA. An Autopsy method for evaluating trauma care. J Trauma 1981; 21:32–34.
22. Committee on Trauma of the American College of Surgeons. Quality assurance in trauma care: appendix G to hospital resources document. Bulletin American College of Surgeons 1987; 72:2:42–47.
23. Shackford SR, Hollingsworth-Fridlund P, McArdle M, Eastman AB. Assuring quality in a trauma system-the medical audit committee: composition, cost and results. J Trauma 1987; 27:866–875.
24. Detmer DE, Moylan JA, Rose J, et al. Regional categorization and quality of care in major trauma. J Trauma 1977; 17:592–599.
25. Van Wagoner FH. Died in hospital. a three year study of deaths following trauma. Read before the Twentieth Annual Session of The American Association for the Surgery of Trauma, Coronado, California, October 5–7, 1960.
26. Spain DM, Fox RI, Marcus A. Evaluation of hospital care in one trauma care system. Am J Public Health 1984; 74(10):1122–1125.
27. Morris JA Jr, MacKenzie EJ, Edelstein SL. The effect of preexisting conditions on mortality in trauma patients. JAMA 1990; 263(14): 1942–1946.
28. Morris JA Jr, MacKenzie EJ, Damiano AM, Bass SM. Mortality in trauma patients: the interaction between host factors and severity. J Trauma 1990; 30:1476–1482.

DISCUSSION

DR. ALEXANDER WALT (Detroit, Michigan): I think this is a very well-motivated paper but certainly not an easy one. The thesis advanced merits careful study because it attempts to apply objectivity to clinical impression, for what I sometimes call the Will-of-God syndrome. To do this the authors have created a very sophisticated statistical sieve through which they hope to capture ostensibly preventable adverse events.

The Committee on Trauma of the American College of Surgeons has been an exemplary force in the exercise of stringent self-criticism over the years and in designing strict but attainable standards of practice for the treatment of injured patients. What Dr. Cayten and his group have done, as I see it, is to incorporate the Committee on Trauma's methods and to extend them.

Anytime a surgeon sees a patient, four questions are asked. Is the operation needed at all? How soon does it need to be done? What should be done at the operation? How do we obviate long-term adverse sequelae? These are the questions that this study has been dealing with but I have a few problems with it. Firstly there are some heartening results in that there were very few technical errors, and their conclusion is that in this study at least they no longer made the same sort of errors that they used to make. For example they identified no missed ruptures of the spleen. But a caveat—the numbers are very small.

The second comment is that delays in operating are not clearly defined. The delays seem not to have occurred in abdominal nor in thoracic injuries, but seem to be confined to head injuries. It appears therefore that they still have a big problem in the head injury field, which we know to be a national weakness.

The third group that bothers me—and it is a large group—is labeled "errors in management." I think that you have been rather harsh in your judgment of what constitutes an error in management; for example, in the so-called low-fall group, the vast majority of the patients were 70 and 80 years of age. I will quote from the paper where you state: "The errors involve the inability to prevent and effectively treat complications such as pneumonia, pulmonary embolism, organ system failure in patients that should have recovered from their injuries." This is a vague sort of statement. You are suggesting that unless we prevent pulmonary embolism, unless we prevent pneumonia, unless we prevent organ system failure, we are in error. I think I take issue with this gloomy assessment, regarding it more as an exercise in masochism and self-flagellation.

Some bothersome questions lead from this attitude. If we set an inappropriately high standard, as I think that you have done, might we not then be held unfairly to that unattainable standard? I think that in your zeal to interpret your figures so critically, you have really set yourself, and therefore us, goals that are impossible to reach. Some of your adverse outcomes are often not errors in management as far as I am concerned.

Three questions as I close. How do you propose to translate the findings of this very intricate study into clinical practice? How do we reduce the so-called errors in management? Is the cost of this effort such that we should have this methodology applied on a national level, and is it feasible to do so?

DR. GEORGE SHELDON (Chapel Hill, North Carolina): I think it is important that we evaluate systems of care as well as biologic systems, which is what the authors have done. Of interest during the month of January, when there were 11 deaths attendant on Operation Desert Storm in the Persian Gulf, there were 77 deaths from gunshot wounds in the City of Chicago alone, which of course did not receive much attention.

We have developed trauma systems, and, as alluded to by the authors, before development of systems through the country, one could almost go into any community and do retrospective autopsy studies and find an approximately 30% preventable death rate. They were often due to rather simple injuries such as ruptured spleens.

Of interest Dr. Miles Irving in Great Britain has conducted a similar study and found that in that country the figures are very much like the United States; when no true system is set up, a number of preventable deaths from relatively simple injuries occurred.

I would also like to ask the authors two questions: First we are in a secondary phase of trauma systems in this country. A number of hospitals are opting out of level 1 commitment because of economic reasons. We see that when revocation of motorcycle helmet laws occurs death rates go up.

Have you seen such a mortality–morbidity trend incident to the discontinuance of trauma centers?

The last question is, I am a bit puzzled that you have 13,000 cases even, which must be an arbitrary place to stop.

DR. H. CHAMPION (Washington, DC): Documentation of the need for and impact of trauma systems has frustrated many. The problem has been and is in controlling for case mix and different severities in injured patient populations.

The TRISS methodology was introduced as a method to try to address some of these problems, but this study, among others, has indicated its frailty in application to quality assurance and for preventable death identification.

Other potential applications of severity scores will ultimately involve cost reimbursement and resource allocation as well as allowing for valid scientific comparison of patient populations of multiple trauma patients over time or between different institutions.

Cayten et al. have taken another important step along the path that will lead us, it is hoped, to more robust methods for quality assurance and accountability in trauma care, bringing at least the spector of objectivity to peer review. And I would stress that the TRISS methodology in some respects is an illusion in this respect. As emphasized by President Zeppa in his address, there is plenty of room for improvement in trauma care.

A number of questions are prompted by this study. First of all "preventable deaths" can clearly occur with predicted mortality rates of greater than 50%. Perhaps Dr. Cayten could comment on why the study was limited at this level. And has he concluded that there are other thresholds that would be more advantageous to study?

The TRISS methodology is only valid when bins are of at least 100 patients. Although I have the manuscript, I could not quite ascertain whether the bins were large enough for adequate statistical comparisons, and whether the W statistic was used in addition to *z*.

There is clearly a difference between statistical significance and clinical significance, and therefore the TRISS methodology does need to be led by a peer review process. Was "preventable death" clearly defined at peer review or just from the statistical methodology?

The average preventable death rate of 13% is actually quite high when compared with the preventable death rates found in mature trauma systems. The average preventable death rate in some 20 or 30 studies in nontrauma systems is about 19.2%. When a trauma system is implemented, trauma centers show a preventable death record of 2% to 3%, and nontrauma centers in the trauma system will show a preventable death rate of about 5% to 8%. These figures are confirmed by data from our own institution working in conjunction with Parkland South Western University Dallas and with UCSD.

The study presented here was done in hospitals that were both trauma centers and nontrauma centers. Are patients being inappropriately triaged in the trauma system, and how does that influence the findings?

We have shown that autopsies are extraordinarily important in identifying the total extent of injuries from patients who died early after injury. This study was likely to prejudice itself if good autopsy data were not available, particularly in those patients who died within the first 48 hours of injury.

In closing we certainly take the important findings of this study as we proceed to start analyses of the MTOS data base of 170,000 patients. We regard this study as important in the continued quest for indices of severity and methods of their application that are robust enough for the applications against which they are being called to perform.

DR. C. GENE CAYTEN (Closing discussion): I agree with Dr. Walt that we probably were a little tough on ourselves in indicating the "possible preventability" of deaths in the elderly group of patients. We did indicate quite distinctly that these deaths were "possibly preventable" deaths and not "frankly preventable" deaths, the term that Dr. Shackford used for one group in a study from San Diego.

We focused on the question, what can we do to be more aggressive in the management of these elderly patients? As Dr. Walt pointed out, many of the preventable death patients were those who developed pulmonary emboli after hip fractures. We believe that preoperative physiologic assessment of these patients, early surgery, anticoagulation, and early ambulation should have a strong effect on minimizing these deaths.

Studies such as this are not easily replicated because of the expense. We had nurses meticulously abstracting the data from medical records. At present we have a grant from the Centers for Disease Control to look into the linkage of the uniform hospital discharge data set that each hospital provides to their state health department to the prehospital ambulance run reports to generate probabilities of survival on injured patients. Such linkage might enable preventable death studies to be done on a less costly basis than this grant-funded project.

Dr. Sheldon, unfortunately, this study was not a population-based study. Therefore it was very difficult to use it to assess a particular trauma care system.

Both Dr. Sheldon and Dr. Champion addressed our specific preventable death rate. We should not attempt to compare preventable death rates. The ways in which these studies have been performed are very different; with different methods of choosing the sample, different types of members on the review panels, and different methods of arriving at consensus. Also if you really look at the mathematics of the proportion of preventable deaths, in the denominator is the unsalvageable deaths plus the preventable deaths. Systems differ in the proportion of unsalvageable patients that they get through the emergency department doors. In many rural areas patients continue to die before they reach the hospital, either at the scene or en route. In other areas these same patients reach emergency departments with minimal vital signs but are still unsalvageable; thus changing the preventable death rate. We really need to be looking at "efficacy rates."

Dr. Champion, we did not use the W statistic. In terms of trauma centers *versus* nontrauma centers, of our eight hospitals, three were level 1 trauma centers and three of the other hospitals were equivalent of level 2 trauma centers. Most preventable death studies have lumped the level 1s and level 2s together. We did not find a statistically significant difference between our three level 1s and the other five hospitals. Also we have not built into the area systems protocols the transfer of geriatric patients to trauma centers.

We do agree with the concern that autopsies were not generally available for this study. The uniform availability of autopsy data should definitely improve the validity of preventable death studies.

Immunomodulation of Kidney and Heart Transplants by Anti-idiotypic Antibodies

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To explore the possibility that circulating HLA antigens from the graft and anti-anti-HLA (anti-idiotypic) antibodies influence the long-term survival of renal and cardiac allografts, analysis of 330 renal allograft recipients and 174 recipients of cardiac allografts was conducted. Anti-donor-HLA antibodies (Ab1) present before or after transplantation are associated with graft failure, whereas irrelevant anti-HLA antibodies had no impact on actuarial graft survival. Ab1 may be uncovered by dissociation of immune complexes and depletion of soluble antigens with monoclonal antibody-coated magnetic beads. Of the 421 sera tested from 65 heart recipients, 97 showed Ab1 before depletion and 178 after depletion; similar rise in positive sera was seen in 39 renal transplant recipients. Three distinct patterns of appearance of Ab1 and Ab2 (anti-Ab1 antibody) were recognized. Patients with cyclic variations of Ab1 in association with Ab2 had 100% graft survival, whereas patients with cyclic variations of Ab1 but no detectable Ab2 had 2-year graft survival of 36% for kidneys and 71% for hearts. Presence of Ab1 in all sera after transplantation led to 47% and 56% 2-year renal and heart allograft survival, respectively.

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GREAT PROGRESS IN transplantation is emphasized by more than 80% cadaveric kidney allograft survival and more than 75% cardiac allograft survival for more than 1 year at major centers. Long-term graft survival for both kidneys and hearts continues, however, to be impeded by chronic rejection. There is no effective therapy for progressive renal allograft dysfunction that may begin 1 to 2 years after transplantation,[1,2] nor for the accelerated arteriosclerosis, which is a manifestation of chronic vascular rejection of cardiac allografts and occurs in about 40% of the patients who retain their grafts for longer than 1 year.[3,4] It remains unclear whether chronic rejection is a distinct entity with a unique cause or whether it is the end result of successive episodes of acute cellular rejection.[2]

Because the rate of chronic kidney loss is lower in recipients who are well matched with their donors for human leukocyte antigen (HLA) antigens than in poorly matched patients,[1] late transplant failure is thought to be caused by patients' immune responses against the disparate HLA antigens of the graft. Similar reasoning applies to cardiac transplantation despite little direct evidence, because the development of cytotoxic anti–B-cell antibodies after transplantation has been shown to correlate with irreversible graft failure.[5] It is still uncertain, however, whether the cytotoxic antibodies detected in the patients' sera are specific to the donors' HLA antigens and whether circulating immune complexes are present in patients undergoing chronic rejection of kidney or heart allografts.

In previous studies[6,7] we have shown that allosensitization through transfusion, transplantation, or pregnancy leads to the development of anti-HLA antibodies (Ab1), which in turn stimulate the production of anti-anti-HLA autoantibodies (Ab2). Such anti-idiotypic, anti-anti-HLA antibodies block the cytotoxic activity of anti-HLA antibodies *in vitro.* Their presence seems to be associated with prolonged survival of organ allografts, accounting for the beneficial effects of pretransplant donor-specific blood transfusions in renal transplantation.

The network theory of Jerne[8,9] predicts that autoimmune responses to cell idiotypes form the basis of immunoregulatory network systems, in which homeostasis is preserved through a functional assembly of idiotype–anti-idiotype interactions. A new organ and its antigens may, based on this theory, only perturb the system until

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it reaches a new steady state. Immunoregulatory circuits based on idiotypic interactions between and within the T- and B-cell compartment therefore may determine the type, duration, and magnitude of any immune state. Anti-idiotypic antibodies have been shown to suppress or enhance the relevant idiotypes. Suppression may occur when Ab2 blocks the differentiation of B cells producing Ab1, or of T cells required for the activation of Ab1-producing B cells, or when anti-idiotypic antibodies engage subsets of suppressor T cells that express the relevant anti-idiotype.[10–13]

The major obstacle to organ transplantation, other than availability of cadaveric donors, remains the difficulty of providing suitable, cross-matched negative grafts to highly sensitized patients and our inability to manipulate the immune response to positively influence long-term renal and cardiac allograft survival. We have explored in the present study the possibility that circulating HLA antigens from the graft and anti-anti-HLA (anti-idiotypic) antibodies influence the long-term survival of renal and cardiac allografts.

Materials and Methods

Renal and Cardiac Allograft Recipients

The study includes 330 recipients of cadaver renal allografts, all of whom received cyclosporine-based triple immunosuppressive therapy and were treated for rejection episodes with either increased doses of steroids or OKT3 antibody (Ortho Pharmaceuticals), or both. We also evaluated 170 recipients of orthotopic cardiac allografts, all of whom received cyclosporine-based triple immunosuppression therapy and were treated for rejection with increased doses of steroids or OKT3 (Ortho Pharmaceuticals).

All patients were typed for HLA antigens and had been monitored after transplantation for development of anti-HLA antibodies in the sera. Rejections were confirmed either by percutaneous renal biopsy or by an endomyocardial biopsy. To ensure the completeness of at least 1 year of follow-up studies, patients with functional grafts who had been transplanted after April 1989 were not included. The study was restricted to patients who, at the time of transplantation, showed no anti-donor HLA antibodies in the serum and from whom four quarterly sera were obtained within the first year after transplantation. The patients dying of surgical complications or of infections within the first month after transplantation were not included in this study.

Statistical Analysis

Graft survival rates were computed by the actuarial method, and Student's t test was used for a statistical comparison.[14] The BMDP Statistical Software Package was used for all analyses.[14] Duration of transplant survival was computed from the date of transplantation to the date of recipient's return to dialysis in renal transplantation or to the time of patient death or retransplantation in cardiac transplantation.

Tissue Typing (HLA-A, B, C, and DR)

Recipients and donors were typed for HLA-A, B, C, and DR antigens using purified T-cells for detection of class I antigens and purified B-cells for HLA class II typing. Typing was performed by complement-mediated micolymphocytotoxicity assay using 5,6 carboxyfluorescein diacetate and ethidium bromide for discriminating between viable and dead lymphocytes. A set of monospecific anti-HLA sera from our laboratory together with HLA typing trays from One Lambda, Inc. (Los Angeles, CA) were used for the tissue typing.

Direct Cross-Matching of Recipient Serum with Donor Lymphocytes

Serum samples from renal transplant candidates, collected monthly, within a 6-month period before transplantation, were cross-matched directly with the donor's lymphocytes. The final cross-matching included serum obtained at the time of transplantation. Patients with a negative cross-match were considered suitable candidates for transplantation regardless of the number of HLA antigens for which the donor was mismatched. None of the patients included in this study had a history of antibodies against HLA antigens present in the graft, as determined by computer search of each recipient's immunologic file.

Evaluation of Anti-HLA Antibodies

Patients were screened for the presence of lymphocytoxic antibodies against all established HLA-A, B, C, DR, and DQ antigens represented on HLA reference panel, consisting of lymphocytes from 70 unrelated individuals. An automated Leitz microscope operated by an Arden System Computer (Arden Systems, Los Angeles, CA), was used to identify the antibody specificities of each serum sample. Assignment of an anti-HLA-antibody specificity was based on correlation coefficients of at least 0.7 with the respective HLA antigen. The program automatically transfers into the patient's file the specificity of the antibody detected and frequency of positive reactions exhibited by the serum on the T-cell panel and on the panel of B lymphocytes.[6]

Immunodepletion of Soluble HLA-Antigen from Patient's Sera

Human leukocyte antigen class I and class II antigens were removed from the sera using immunomagnetic beads as previously described.[15] For HLA class II antigen depletion, we used Dynabeads HLA Cell Prep II (Dynal Inc., Great Neck, NY) a 4.5-μm supermagnetic bead coated with a monoclonal antibody against a monomorphic epitope present on HLA-class-II molecules. Human leukocyte antigen class I antigens were adsorbed on Magnisort-M chromium dioxide particles (Dupont Corporation, Wilmington, DE) to which goat anti-mouse immunoglobulin was coupled. Before use the particles were coated with a murine monoclonal antibody B9.121 (Pel Freeze, Brown Deer, WI) that reacts with a common epitope of all HLA class I antigens. Soluble HLA class II and class I antigens were coupled to beads and they were incubated for 1 hour at 4 C with continuous gentle mixing. The beads then were removed and the antigen-depleted serum was tested in several dilutions for reactivity with T and B lymphocytes from the HLA reference panel. The nondepleted counterpart of the same serum was tested in parallel to compare the frequency and specificity of lymphocytotoxic antibodies.

Depletion of Immune Complexes from Patient's Sera

Tosyl-activated magnetic beads obtained from Dynal Inc. (Great Neck, NY) were coupled with purified C1q (150 mEq/mL) at 1:1 ratio as described by the manufacturer. The coupling efficiency was monitored by indirect immunofluorescence using fluorescinconjugated goat anti-human C1q antibody (Cappel, Organon Techniqua Corp., PA). The ability of C1q-coupled beads to remove immune complexes was ascertained before the demonstration of their ability to remove Ig complexes efficiently after incubation with 100 to 1000 μg of heat-aggregated purified human IgG (Jackson Immuno Research Laboratories, Malvern, PA).

Determination of Anti-Anti-HLA Antibodies (Ab2) in Recipient Sera

The presence of anti-idiotypic antibodies in recipient serum was ascertained by lymphocytotoxicity inhibition studies as we have previously described.[6] To detect anti-idiotypic antibodies (Ab2), we tested directly in Terasaki tissue typing trays the capacity of sera that contained no anti-HLA antibodies (putative Ab2) to inhibit Ab1 (anti-HLA antibodies) present in serum samples obtained from the same patient at another time. As a control the autologous serum sample obtained before transplantation, and before exposure to the Ab1-inducing antigen, was tested for blocking activity. All sera previously depleted of soluble antigens by magnetic immunoaffinity were screened for anti-HLA antibodies on the 70-cell HLA reference panel before being tested for Ab1 or Ab2 as previously described.[6] The reaction was read using 1 μL of ethidium bromide for staining the dead cells. The titer of the serum was established from the highest dilution in which 100% target cell lysis occurred. Sera depleted of soluble HLA antigens were tested for Ab2. Serial dilutions of Ab1 (1 μL per well) were mixed with an equal volume of HLA-antigen–depleted test serum (putative Ab2); the mixture was incubated for 1 hour at 22 C. Target cells (5×10^3/well), rabbit complement (5 μL), and ethidium bromide (11 μL) were added to the wells in sequence, at 30-minute interval. Reaction was read immediately after the addition of ethidium bromide.

Blocking by putative Ab2 of onefold, twofold, or more than threefold dilutions of Ab2 was considered indicative of the presence of Ab2. Specificity controls consisted of sera with antibodies against the same or against a different HLA antigen.

Biopsies

Renal biopsies were performed only for specific indications, which included any significant deterioration in renal function that could not be explained by technical problems (85% of patients studied had at least one biopsy). Biopsies were usually performed within the first 3 months of transplantation or 1 year or more after transplantation. Cellular or interstitial rejection was defined morphologically as focal or diffuse interstitial infiltrates of lymphocytes, plasma cells, and monocytes associated with interstitial edema and tubulitis. Humoral or predominantly vascular rejection was defined as active endovasculitis of arterial intima by immunofluorescence studies. Chronic rejection was subdivided into two types; primarily cellular (tubulointerstitial) or predominantly vascular (humoral) types.

Cardiac allografts were examined by biopsy once a week for the first 3 weeks, every 10 days over the next month, and then every 3 weeks for another 6 weeks. Tissue biopsies then were obtained monthly to the end of the first year, and additional biopsies were performed when rejection was suggested either clinically or on the basis of noninvasive tests. Myocardial rejection was defined by findings of mononuclear infiltrate in conjunction with myocyte necrosis.

Results

Effect of Presensitization on Long-Term Survival of Kidney and Heart Allografts

Patients were divided into three groups according to their preformed reactive antibodies (PRA) status: (1) PRA less than 10%; (2) PRA of 11% to 50%; and (3) PRA greater than 50%. Serum was considered positive for anti-HLA antibodies if the antibodies (1) had correlation coefficients greater than 0.7 with a distinct HLA antigen; (2) reacted with more than 10% of the members of the HLA reference panel of T- and B-cell donors; and (3) segregated with

HLA in families of positive panel members.[7] Patients were grouped into the category of anti-HLA antibody producers or nonproducers if they showed antibodies, by this criteria, in at least one of the four sera collected during the first year after transplantation. We compared the actuarial survival of 330 renal and 174 cardiac allografts in patients with antibodies before and after transplantation. In our cardiac allograft recipients, there was a significant difference between patients with PRA less than 10% compared with PRA of 10% to 50% ($p < 0.03$), because the actuarial survival at 4 years was 78% and 49%, respectively (Table 1). In contrast the 4-year survival of renal allografts was 70%, 68%, and 66%, respectively, in patients with PRA less that 10%, 11% to 50%, and >50%. The difference between the two populations is that in renal but not in heart recipients, a cross-match of patient serum with donor lymphocytes is done before transplantation. Confirming this possibility is the finding that analysis of the survival rate in heart allograft recipients presensitized against the donor's HLA antigens showed that 4-year survival was 56%, compared with 70% in patients whose antibodies did not react with the donor ($p < 0.04$). These data suggest that anti-donor HLA antibodies are associated with failure of the graft. There was no difference between the groups of sensitized and nonsensitized recipients in the degree of matching for HLA class I or class II antigens. Although as expected graft survival was lower in patients with positive biopsies compared with those not showing rejection in the first year, the difference between the groups was not significant ($p < 0.09$), suggesting that long-term survival cannot be predicted from the results of first-year biopsies.

Similar observations could not be made in renal transplant recipients because no serial biopsies were done in the absence of clinical indications of transplant rejection. In this study 90% of the patients on whom biopsies were performed had evidence of cellular or humoral rejection, whereas 10% showed other findings such as cyclosporine toxicity, *de novo* or recurrent disease, or "normal" biopsy. Four-year graft survival could not be predicted from the results of first-year biopsy; patients not requiring biopsy appeared to fare better.

TABLE 1. *Effect of Presensitization on Long-Term Survival of Kidney and Heart Allografts*

Specificity and Degree of Presensitization	No. Kidney Allografts (n)	No. Heart Allografts (n)	4-yr Actuarial Graft Survival (%)	
			Kidney	Heart
Presensitized				
With anti-donor Ab	8	5	34	56
Without anti-donor Ab	63	35	72	76
PRA				
<10%	259	130	70	78
11–50%	56	29	68	49
>50%	15	15	66	84

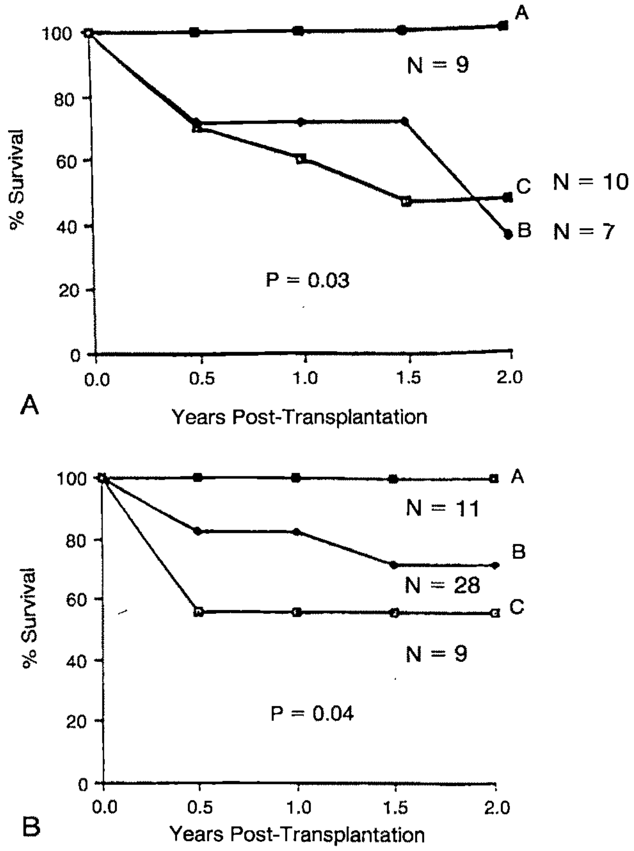
TABLE 2. *Frequency of Anti-HLA Antibodies in Sera from Patients with Chronic Rejection and with Stable Renal and Cardiac Graft Function after 4 Years*

Outcome of the Graft	No. of Patients	No. Sera with Anti-HLA Antibodies/Total Tested	
		Sera not Depleted of HLA Antigens	Sera Depleted of HLA Antigens
Kidney			
Chronic rejection	19	31/76	43/76
Stable function	20	2/82	4/82
Heart			
Chronic rejection	28	49/176	99/176
Stable function	37	48/245	79/245

Development of Anti-HLA Antibodies After Transplantation

Study of post-transplantation sera showed frequently cyclic variations in the level, for example, titer and frequency of anti-HLA antibodies, in both renal and cardiac transplantation, which intrigued us. Our hypothesis was that such variations may be caused by soluble HLA antigens from the graft or by anti-idiotypic antibodies that block the cytotoxic activity of Ab1. The hypothesis was tested by comparing the anti-HLA activity of the sera before and after depletion of HLA antigens. Both in renal and cardiac allografts, the presence of anti-donor HLA antibodies after transplantation was associated with a decrease in long-term allograft survival. Some patients rejected their grafts without developing any anti-HLA antibodies, however, whereas others showed lymphocytotoxic antibodies with no distinct antibody specificity. Table 2 shows that of the 421 sera tested from 65 heart transplant recipients, 97 showed anti-donor HLA antibodies before depletion of HLA antigens; the corresponding fraction was $^{178}/_{421}$ after depletion of soluble antigen. The data obtained in 39 patients with renal transplants show that an increased proportion of sera from patients with chronic rejection have anti-HLA antibodies after depletion of soluble HLA antigens (57%) compared with the results obtained before depletion (41%). Some sera became positive only after immunodepletion of HLA antigens. Sera from patients tolerating allografts for 4 years reacted only at a rate of 2% with donor lymphocytes before depletion; 5% were positive after depletion of soluble HLA antigens. The difference between the frequency of antibody-positive sera in patients who rejected and patients with successful grafts was highly significant both in renal and cardiac allotransplantation ($p > 0.0001$). The dissociation of immune complexes through immunoaffinity depletion of soluble antigens resulted not only in an increase in the frequency of panel-reactive antibodies but also in a better definition of their anti-donor specificity, thus allowing this determination to have a prognostic value both in renal and cardiac transplantation. Patients who had anti-HLA antibodies in at least one serum were tested for Ab2. Assays for Ab2 were performed only after all sera were depleted of soluble HLA antigens. A total of 26 renal graft recipients and 48 heart allograft recipients were informative for this study (Fig. 1A and 1B). They showed anti-donor Ab1 in at least one of their post-transplantation sera. Three distinct patterns were recognized: (1) patients with cyclic variations of Ab1 in association with the development of Ab2; (2) patients with cyclic variations of Ab1 but no detectable Ab2; (3) patients with Ab1 in all sera obtained during the first year after transplantation. The 2-year survival of kidney and heart allograft recipients with Ab2 was 100%. The survival of the graft in patients with cyclic variations of Ab1, unrelated to Ab2, was 36% in kidney recipients and 71% in heart recipients. Heart and renal grafts with consistent levels of Ab1 in all post-transplantation sera showed a survival rate of 56% and 47%, respectively.

It therefore appears from this study that the presence of immune complexes has a positive association with a poor outcome. Studies of the kinetics of antibody formation after renal or cardiac transplantation indicated that patients undergoing chronic rejection show no Ab2, whereas patients who maintain stable allograft function in spite of developing Ab1 produce anti-idiotypic antibodies (Ab2). Because these patients showed no Ab2 before transplantation, it recalls that Ab2 was produced in response to anti-graft Ab1, and that the presence of Ab2 is indicative of an excellent prognosis in both renal and cardiac recipients.



FIGS. 1A and B. (A) Actuarial survival of renal allografts in 26 recipients who (line *A*) showed cyclic variations of Ab1 in association with the development of Ab2; (line *B*) showed cyclic variations in Ab1, but no detectable Ab2; or (line *C*) showed Ab1 in all sera obtained during the first year after transplantation. All sera collected in the first year after transplantation were depleted of soluble HLA antigens before Ab1 or Ab2 assays. (B) Actuarial survival of heart allografts in 48 recipients who (line *A*) showed cyclic variations of Ab1 in association with the development of Ab2; (line *B*) showed cyclic variations in Ab1, but no detectable Ab2; or (line *C*) showed Ab1 in all sera obtained during the first year after transplantation. All sera collected in the first year after transplantation were depleted of soluble HLA antigens before Ab1 or Ab2 assays.

Discussion

Despite the progress that has been achieved in transplantation, the cause and mechanism of chronic allograft rejection remains unclear. It is suggested that humoral antibodies are a major factor contributing to its development and progression, yet the nature of the antigen that elicits the production of such antibodies is still unclear. Although most rejections are T-cell mediated, humoral antibodies also can be involved, particularly in immediately vascularized organ allografts whose vascularization is of donor origin. When anti-MHC (major histocompatibility complex) antibodies encounter donor MHC antigens on endothelial cells of vessel linings, the endothelial cells become susceptible to antibody-mediated damage. Although high concentrations of anti-donor MHC antibodies may lead to hyperacute rejection of kidney allografts, certain anti-MHC antibodies may act as enhancing antibodies, by blocking T-cell access to the target.

We were stimulated initially to investigate this question in relation to beneficial effects of blood transfusions in prolonging renal allograft survival.[16–18] Despite the fact that blood transfusions are a well established source of alloimmunization, they are known to condition the recipient for graft acceptance. We focused our efforts on a better understanding of the paradoxical effect of blood transfusions[6] and of fetal tolerance in pregnancies.[19] The most salient conclusion of these studies was that anti-idiotypic autoimmune responses down-regulate the immune response to HLA alloantigens. This led us to suggest that the basic mechanism that secures maternal tolerance of the semi-allogeneic fetoplacental unit, and by implication the preservation of MHC polymorphism in tandem with T-cell alloreactivity, resides in the development of anti-idiotypic autoimmunity to MHC.[20,21]

In other early studies from our laboratory, we showed that patients undergoing chronic rejection of renal allografts developed lymphocytotoxic anti–B-cell antibodies.[22] Preferential reaction of the antibodies with B rather than with T lymphocytes could be explained by the fact that the level of expression of HLA antigens is higher on B than on T lymphocytes, or by the fact that the antibodies recognized HLA class II antigens that are expressed on B, but not on resting T cells. Despite these findings the pattern of reactions that were found did not permit the characterization of such antibodies with respect to the antigen that they recognized, which could have been B cells, kidney cells, or endothelial cells, as suggested by the eluates obtained from chronically rejected kidneys. It is possible that graft atherosclerosis in cardiac allografts is also related, at least in part, to damage of vascular endothelium by anti–B-cell antibodies.[5] Lesions in accelerated atherosclerosis in cardiac allografts are somewhat reminiscent of immune-mediated vascular damage that accompanies chronic kidney allograft rejection in humans. Chronic rejection of kidneys and hearts has been thought to be triggered by immune mediators, such as cytokines produced by T cells sensitized to non-MHC, rather than by MHC antigens. Such hypotheses were supported by the observations that patients regrafted with a kidney carrying HLA antigens, present in the first graft, did not have a lower graft survival rate.[23] Surgical trauma itself, with resulting inflammation, processing of graft antigen by monocytes, and release of soluble antigens in the circulation may eliminate these events. The analysis of long-term renal and cardiac allograft survival emphasizes that understanding of the nature of antibodies developed by patients with chronic rejection is critical to establishment of efficient strategies for preventing, monitoring, and treating chronic rejection. Our data show that the 4-year survival of kidney allografts was 66% to 70% in the absence of a positive cross-match, regardless of the level of PRA detected within 6 months before transplantation. Similar observation was made in patients receiving cardiac allografts who did not exhibit anti-donor antibodies. We further established that the development of antibodies after transplantation correlates with poor survival of both renal and cardiac allografts. Therefore we conclude that anti-HLA antibodies contribute to rejection. The presence and specificities of anti-HLA antibodies, however, is often difficult to establish because of the release of HLA antigens from the injured graft into the circulation. Antigen depletion experiments demonstrated that sera from patients with chronic renal or cardiac allograft rejection contain complexes of HLA-antigen/anti-HLA antibody. The presence of such complexes explains the cylic variations found in antibody levels after transplantation. Identification of anti-donor HLA antibodies after magnetic immunoaffinity depletion of HLA antigens from post-transplantation sera permits the establishment of the correlation between the presence of free or complexed HLA antibodies and poor long-term prognosis both in renal and cardiac allografts. This procedure permits the early detection of soluble donor antigens and may become a useful diagnostic procedure for the detection of early allograft rejection. Because in this study we found an increased frequency of HLA antigen/anti-HLA antibody complexes in post-transplantation sera from patients who have an abbreviated long-term allograft survival, it is unlikely that soluble HLA antigens from the donor have a protective effect on graft survival, as has been previously suggested.

Because we found HLA antigen–antibody complexes both in renal and cardiac allograft recipients, it appears that HLA antigens are a primary initiator of the humoral immune response. This does not exclude the possibility that tissue-specific antigens also may elicit rejection.

Still unexplained is the fact that some patients tolerate their renal or cardiac allograft in the presence of anti-HLA antibodies. Tolerance in these patients may result from blocking of anti-HLA antibodies either by soluble HLA antigens from the graft or by anti-idiotypic antibodies. The first is unlikely because the majority of patients showing immune complexes develop chronic allograft rejection. The second alternative, in other words, the presence of anti-idiotypic (Ab2) antibodies, is supported by our data; patients forming Ab2 after transplantation had kidney and heart allografts survival of 100% at 2 years, whereas those who had cyclic variations of Ab1 unrelated to appearance of Ab2 showed significant decrease in long-term renal and cardiac allograft survival. These results confirm our earlier data and suggest that anti-idiotypic antibodies may delay rejection and improve prognosis by blocking the production or binding of anti-donor-HLA antibodies to the graft.[24,25]

In conclusion we believe that the study of anti-HLA antibodies in sequential samples of sera after transplantation is a useful prognostic test for long-term graft survival. Measurement of anti-HLA antibodies in sera depleted of HLA antigens by magnetic immunoaffinity provides a possible method for diagnosis of chronic rejection. Finally it appears that an active immune response against mismatched HLA antigens of the graft takes place in all transplant recipients and that the length of allograft survival is influenced by the patients' ability to develop suppressive anti-idiotypic antibodies. Our data emphasize the importance of idiotype-determined regulation of the immune response to HLA as well as the concept that chronic rejection is elicited primarily by mismatched HLA antigens of the graft.

References

1. Terasaki PI, Mickey MR, Cedka M, et al. Overview. *In* Tersaki PI, ed. Clinical Transplants, 1987. Los Angeles: UCLA Tissue Typing Laboratory, 1987.
2. Croker BP, Salomon DR. Pathology of the renal graft. *In* Tisher CC, Brenuer BM, eds. Renal Pathology with Clinical and Functional Correlations. Philadelphia: JB Lippincott, 1989, pp 1518–1548.
3. Billingham ME. Cardiac transplant atherosclerosis. Transplant Proc 1987; 19(Suppl 5):19–25.
4. Uretsky BF, Murali S, Reddy PA, et al. Development of coronary artery disease in cardiac transplant patients receiving immunosuppressive therapy with cyclosporine and prednisone. Circulation 1987; 76:827–834.
5. Hess ML, Hastillo A, Mohana Kumar T, et al. Accelerated atherosclerosis in cardiac transplantation: role of cytotoxic B-cell antibodies and hyperlipidemia. Circulation 1983; 68(Suppl 2):94–101.
6. Reed E, Hardy MA, Benvenisty A, et al. Effect of anti-idiotypic antibodies to HLA on graft survival in renal allograft recipients. N Engl J Med 1987; 316:1450–1455.
7. Reed E, Suciu-Foca N, Hardy MA, et al. Alloantibodies to a non-HLA system and their relevance to transplantation. Transplant Proc 1983; 15:245–249.
8. Jerne NK. Towards a network of theory of the immune system. Ann Immunol (Paris) 1974; 125C:373–389.
9. Jerne NK. Idiotypic networks and other preconceived ideas. Immunol Rev 1984; 79:5–24.
10. Rubinstein LJ, Bona CA. Immune networks. Ann NY Acad Sci 1983; 418:97–108.
11. Bona CA. Idiotypes and lymphocytes. Immunology 1984. San Diego, Academic Press.
12. Bluestone JA, Leo O, Epstein SL, et al. Anti-idiotypic antibodies as immunogenes. Immunol Rev 1986; 90:5–27.
13. Reed E, Lewison A, Suciu-Foca N, et al. The relevance of non-HLA lymphocytotoxic antibodies in renal transplantation. Transplant Proc 1983; 15:1838–1840.
14. BMDP statistical software manual. Vol 2. Berkeley: U. of California, 1988; pp 689–690.
15. King DW, Reed E, Suciu-Foca N. Complexes of soluble HLA antigens and anti-HLA autoantibodies in human sera: possible role in maintenance of self-tolerance. J Immunol Res 1989; 8:249–262.
16. Opelz G, Mickey MR, Terasaki PI. Identification of unresponsive kidney transplant recipients. Lancet 1972; 1:868–871.
17. Feduska NJ, Vicenti F, Amend WJ, et al. Do blood transfusions enhance the possibility of a compatible transplant? Transplantation 1979; 27:35–38.
18. Singal DP, Joseph S. Role of blood transfusions on the induction of antibodies against recognition sites on T-lymphocytes in renal transplant patients. Hum Immunol 1982; 4:93–108.
19. Suciu-Foca N, Reed E, Rohowsky C, et al. Anti-idiotypic antibodies to anti-HLA receptors induced by pregnancy. Proc Natl Acad Sci USA 1983; 80:830–834.
20. Suciu-Foca N, Kohler H, King DW. Anti-idiotypic auto-immunity: a necessity for species survival. Survey of Immunological Research 1984; 3:311–318.
21. Suciu-Foca N, King DW, Reemtsma K, Kohler H. Autoimmunity and self antigens. Concepts Immunopathol 1984; 1:173–189.
22. Hardy MA, Suciu-Foca N, McKiernan C, et al. The development and specificity of anti-B-cell antibodies during humoral rejection in kidney allografts. Transplant Proc 1979; 11:389–392.
23. Opelz G, Terasaki PI. Absence of immunization effect in human retransplantation. N Engl J Med 1978; 299:369–374.
24. Suciu-Foca N, Reed E, D'Agati VD, et al. Soluble HLA antigens, anti-HLA-antibodies, and antiidiotypic antibodies in the circulation of renal transplant recipients. Transplantation 1991; 51:593–601.
25. Suciu-Foca N, Reed E, Marboe C, et al. The role of anti-HLA antibodies in heart transplantation. Transplantation 1991; 51:716–724.

DISCUSSION

DR. A. P. MONACO (Boston, Massachusetts): Thank you. It is a great pleasure for me to discuss this paper. Mark Hardy was my first research fellow, and I have watched his career with great pride and admiration. This paper by Dr. Hardy and his group is a very, very important one.

When Medawar first made his discovery about immunologic tolerance, he based his explanation of this phenomenon on Burnett's theory, that in fact there was actually a clonal deletion of reactive cells, thus producing a nonreactivity as the reason why a graft could survive.

Shortly thereafter a French immunobiologist, Voisen, proposed that another possibility was that there were two reactions that occur when a graft was placed. One was a destructive or cytotoxic reaction, and the second one was what he described as an enhancing or facilitatory reaction. And he proposed the existence of a facilitating antibody, which many people did not accept.

Subsequently over the years, it became more apparent that with every immune response there seemed to be a self-regulating, damping response. And this occurred with almost every kind of immune response that was studied, and it culminated in the Jerne's network theory, and in Jerne's Nobel prize.

In clinical transplantation there were many clinical suggestions for this. All transplanters have had patients who had a violent rejection, that should have lost their grafts, but they got over the severe rejection, and they not only got over it, but they had perfect courses thereafter, like they had been selected out and were protected.

Similar support for this was found in the phenomenon of donor's specific blood transfusion, which was introduced by Salvatierra and his group in California. These patients did very well, but they had a high incidence of rejection. And this rejection could be treated, and then they seemed to be specially protected from future rejections.

So this paper is important because Dr. Hardy and his colleagues have finally given the transplant community, with their work over the last several years, the biologic and immunologic explanation we have been seeking.

And in summary what they have said is that when antidonor antibody is made, it is a bad marker and a bad prognostic sign. If one does not make this antibody, that is a good marker and a good prognostic sign. But even if one does make the antibody, a small group of patients can make a counter-antibody that is protective.

I have three small questions. From your data I assume that the first antibody, antidonor antibody, always is accompanied by rejection. Does it ever come without a clinical rejection phenomenon? Could it be used as a monitor for following patients serially to detect the onset of antigraft activity?

Secondly for the anti-idiotype antibody 2, how tough is it to detect? Could it be used as a serial marker? And is there any way of increasing it?

And then finally if this theory is correct, I would predict that antibody 2 patients who have 100% survival should have had a significant clinical rejection in their course; in fact more clinical rejection, even though they get over it, than the patients who did not make any antibody 1 at all. Is this correct?

This was an excellent paper, and Dr. Hardy and Dr. Reemtsma and their colleagues should be congratulated.

DR. JOSH MILLER (Miami, Florida): I would like to thank Dr. Hardy for the opportunity to preview his paper in an area that I have offhandedly called a subject for immunologic catalysts. The network theory of immunoregulation was promulgated as was mentioned by Jerne almost 20 years ago, and subsequently he received the Nobel prize for it. It has had numerous clear-cut substantiations in experimental animal models, including transplantation.

In the early 1970s, we had the good fortune to describe antibody, that is immunoglobulin, inhibition of T cell proliferative responses to donor antigen presenting cells in mixed lymphocyte cultures in human, in well-adopted kidney transplant recipients. Although in many of these patients, the antibodies were against the donor alloantigens, there was an immunoglobulin repertoire in a few patients in which binding with the donor cells did not remove the MLC inhibitory property, and this hinted at the presence of antibody 2, rather than the enhancing antibody that was described by Volsin and Kaliss in the late 1950s.

In the late 1970s, a much more eloquent scheme was described by Binz and Wigzell in rats, who investigated antibodies directed at T cell epitopes, and subsequently were shown by others to be directed at the polymorphic determinants of the T cell receptor; in other words, an autoregulatory antibody that might prove to be the magic bullet in immunoregulation.

Now Dr. Hardy and his colleagues have very insightfully carried us further into this approach by showing the more classical anti-idiotype effect, an effect on B cell products, that is, antibody modulating a response to cytotoxic anti-donor antibodies.

Now the problems presented to the immunologic catalist are definitive demonstration of this singular antibody by clonal technology, and Dr. Hardy's sophisticated group is most capable of doing this, and may well have done it already.

Therefore I have three questions of many posed by this most provocative paper.

One: Can you clone the B cells making antibody 2?

Two: Do you have evidence in patients who have this antibody 2 waxing and waning that antibody 3 might be present? Tools can be used to demonstrate this now, such as new solid-phase immunoassays.

Three: Are there specific patient groups that can be segregated by responder status? In other words can you correlate a positive concordance with MHC type using histocompatibility typing studies, of patients that will develop antibody 2 or not?

DR. N. LEMPERT (Albany, New York): Dr. Hardy and his co-workers have taken their original thoughts on blocking antibody, and after careful *in vitro* studies have now carried out an important clinical study. It is a study that has broad implications for both the pretransplant selection of patients as well as the postoperative immunosuppression of both cardiac and renal transplants.

I have three questions. Dr. Hardy, do you believe that the development of anti-idiotypic antibody is generated by transfusions or the transplant? And if so should we be treating patients lacking anti-idiotypic antibodies with donor cells in a randomized blinded study in the early post-transplant period?

Secondly because the majority of patients lacking anti-idiotypic antibody lost their grafts during the first 3 to 6 months, have you changed your immunosuppressive regimen to accommodate these patients?

And finally based on this paper, and your prior publications, it would appear that patients with pretransplant anti-idiotypic antibodies should be given preferential points in the selection for transplantation. Although this idea flies against the current trend toward equality, it certainly would provide better graft survival in renal and cardiac transplant patients, and a more economical use of an increasingly scarce donor resource.

DR. BILL GAY (Salt Lake City, Utah): From the outset the philosophy of our cardiac transplant program has been to aggressively treat very early forms of rejection, our philosophy being that if we could suppress rejection early, perhaps there would be less in the way of late rejection.

In addition to the standard immunosuppression of steroids, azathioprine and cyclosporine, we have used antilymphocyte globulin early on in our experience, and later switched to the monoclonal antibody OKT3 in three separate clinical trials: 10 days, 14 days, and 21 days in length.

We had good results with 10 days, better results with 14 days of OKT3 suppression, but at 21 days we began to see anti-mouse antibodies develop with at least the implication that there were anti-HLA antibodies developing when the OKT3 levels became suppressed. Our long-term results seemed to verify this interpretation, in that a number of the grafts of those patients in the latter OKT3 trial were lost and had to be replaced.

Therefore we would agree enthusiastically that specificity of the targeting of antirejection therapy is the wave of the future, and certainly monitoring of this response is appropriate.

I have two questions for Dr. Hardy. First you implied that there are good antibodies and bad antibodies. Is there information that perhaps bad antibodies through either treatment or through the graft remaining intact can be changed to become good antibodies? Does prolongation of graft survival make the graft less responsive to the bad antibodies?

And secondly what new agents are you using in the laboratory to investigate the phenomenon that you reported on?

DR. JOSEPH E. MURRAY (Boston, Massachusetts): This is a most satisfying paper. I have always been puzzled by the inexplicable vagaries in the immunoregulatory systems. Even in the classic paper of Billingham, Brent, and Medawar, only a small percentage of the mice could be made tolerant. In some strain combinations, tolerance was impossible to achieve.

I recall observing whole mounts of some of our rejected dog kidney allografts and noting the patchy rejection patterns. Frequently most of the kidney would look normal, with only 20% or so having gross rejection. Another puzzle is the unexpected rejection in well-functioning allografts.

I like the last slide, "*In Vivo Veritas.*" Man is the ultimate experimental model. This paper illustrates that clinicians can be scientists and as productive as basic investigators working at the bench.

DR. MARK A. HARDY (Closing discussion): Dr. Murray, thank you very much for your comment about the surgeons being scientists. An additional explanation in this particular case, is that the surgeons collaborated with scientists, and Dr. Suciu-Foca has contributed many of the ideas and certainly a lot of the work in this area, as did the immunogeneticists. So it is a close collaboration between surgeons and basic scientists that can lead to this kind of work.

Dr. Monaco, thank you for everything you have taught me for many years. And now I thought you would be kinder and ask simpler questions, but I guess you did not want to do that.

The answer to your question, as to whether the antibody appearance of Ab1 correlates with biopsies: It does only sometimes, and it is a good thing that you cannot come up here and ask me why.

We have examined this question particularly in heart transplants, where there are serial biopsies, and the appearance of Ab1 does not correlate directly with biopsy results. This is not particularly surprising because the appearance of Ab1 can be caused by just shedding of antigens, and you may not see the rejection phenomenon in the biopsy, which is a relatively gross and late phenomenon.

Can Ab2 be used as a monitor? Yes, it can, and that is what we are doing. We feel much better about those patients who have Ab2 because they actually go on to retain their grafts and do not cause any problems.

I think Dr. Miller asked me whether there were variations in Ab2, and indeed there are variations, but we cannot detect Ab3 because it is similar to Ab1. I think that some of the newer techniques will allow us to distinguish the difference between Ab1 and Ab3.

Dr. Monaco predicted that there might be a very severe rejection before the appearance of Ab2. That, unfortunately, Tony, also is not true. As you know, and as I learned in your laboratory, Mitchinson once postulated many years ago, when I was a youngster, that you can induce unresponsiveness with very low doses of antigen as well as with very high doses.

In the majority of our patients, it appears that the stimulation of Ab2 is with extraordinarily low doses of Ab1 that we cannot detect, either by rejection or by anything else, and we suspect that this due to shedding of antigen. There is probably a micro-micro-rejection going on to account for that.

Dr. Miller asked if we could clone the B cells. Yes, they can be cloned, both the B cells and T cells. Unfortunately they lose their ability to make antibodies, so it is not terribly useful. Dr. Suciu-Foca's laboratory is working very hard on cloning the T cell receptor and is achieving that, which should be most productive.

There is a correlation between the responder status and the Ab2 production. It occurs particularly with DRB16.1 as a nonresponder or a producer of Ab2. So we have found that to be true.

To answer Dr. Lempert's question on transfusions, they are indeed important. It is a double-edged sword. We did look at this very carefully and published the results, last year in the *New England Journal of Medicine,* where some patients who have had donor-specific transfusions develop Ab2, but not all. Some develop Ab1 and go on to chronic rejection.

I think one of the major explanations for the beneficial effect of transfusion is the appearance of anti-idiotypic antibodies. I think that is why it works. I think that in terms of how we should treat our patients in the future, our thought is not to give them donor cells but to vaccinate them, if you will, with the appropriate immunoglobulins.

To answer Dr. Gay's question, we have done that type of experiment in rats. Dr. Wasfie presented our work last year at the Society of University Surgeons, in which we were able to induce prolongation of heart allograft survival in rats by inoculation or vaccination, if you will, with the appropriate IgG and adjuvant.

There are many laboratory experiments that can be done. The Ab1 that you alluded to cannot be changed. Conversely one can produce an anti-antibody, and that is Ab2. And, as you know, when you remove the graft that is badly rejected, the Ab1 titer rises rapidly. So Ab1 is hidden partially in a rejected graft as well.

The question you asked me about the prolonged treatment with OKT3 leading to poor graft survival in patients is probably due to viral or other infections. I do not think that there is any cross-reactivity between mouse antibodies and HLA in this situation. However OKT3 could theoretically suppress the production of Ab2, so it may be unfavorable to overimmunosuppress patients from an immunologic point of view, not just from the point of view that we have always worried about, that is, the increased incidence of infections.

I hope I have answered some of the questions that were asked, but I am sure I have not answered them all. Thank you all for your provocative discussion.

Laparoscopic Cholecystectomy

Experience With 375 Consecutive Patients

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Three hundred seventy-five consecutive patients underwent laparoscopic cholecystectomy from September 1989 to January 1991. Three hundred forty-one (91%) presented on an elective basis, and the remaining 34 patients (9%) were admitted for acute cholecystitis (24), gallstone pancreatitis (9), and cholangitis (1). Of the 375 patients, 20 were converted to laparotomy and cholecystectomy, for an overall success rate of 95% for patients undergoing laparoscopic cholecystectomy. Three hundred nineteen patients (90%) were discharged within 24 hours of surgery. Operative cholangiography was completed in 141 patients, showing choledocholithiasis in five (managed by postoperative endoscopic retrograde cholangiopancreatography [ERCP] in 4, common bile duct exploration [CBDE] in 1). Two retained stones (0.9%) were detected in 214 patients not undergoing cholangiography. Three patients (0.8%) were reoperated on because of perioperative complications. Overall morbidity for patients undergoing laparoscopic cholecystectomy was 3.5%. Major complications (0.6%) included a single common hepatic duct injury and a delayed cystic duct leak at 10 days. Minor complications occurred in 11 patients (2.9%). The single perioperative death (0.3%) was due to a myocardial infarction on postoperative day 3, after an otherwise uncomplicated laparoscopic procedure. Laparoscopic cholecystectomy appears to offer significant advantages to patient recovery, and these data suggest that it can be performed with an efficacy, morbidity rate, and mortality rate similar to those of open cholecystectomy.

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THE USE OF laparoscopy to perform general surgical procedures has stimulated extraordinary interest from the medical community. Initial reports indicate that laparoscopic cholecystectomy may offer a significant number of advantages over open cholecystectomy for the treatment of symptomatic gallbladder disease.[1-4] These early reports that indicated substantial reductions in hospitalization and recovery periods, decreased postoperative discomfort, and improved cosmesis have generated enthusiasm from both patients and physicians. This initial enthusiasm, however, has been tempered by concern over the incidence of major complications during laparoscopic cholecystectomy. Unfortunately data evaluating the safety of this new procedure have, for the most part, been limited to series reporting on relatively small numbers of patients.[1-5] Definitive recommendations on the role of laparoscopic surgery must await analysis of more extensive series. Our experience with 375 consecutive patients undergoing laparoscopic cholecystectomy at a single institution is therefore reviewed.

Materials and Methods

Four hundred three patients were operated on for symptomatic gallbladder disease from September 1989 to January 1991 at the University of Maryland Medical System. Twenty-eight patients were scheduled directly for open cholecystectomy because of either lack of surgeon experience in laparoscopic-guided techniques or the presence of a contraindication to laparoscopic cholecystectomy. Currently observed absolute and relative contraindications to laparoscopic cholecystectomy include those listed in Table 1. The remaining 375 patients underwent laparoscopic cholecystectomy as the primary procedure and constitute the study population. Informed consent was obtained from all patients, at which time the nature of the procedure and the potential for conversion of the laparoscopic approach to an open cholecystectomy was explained.

The mean age in all 375 patients was 47 ± 0.8 years, with a range of 16 to 94 years. Two hundred fifty-nine of the 375 patients were women (69%) and 116 were men (31%), thereby yielding a female/male ratio of 2.2:1.

All patients presenting for treatment of symptomatic gallbladder disease underwent routine history, physical

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TABLE 1. *Contraindications to Laparoscopic Cholecystectomy*

Absolute Contraindications	Relative Contraindications
Sepsis	Previous upper abdominal surgery
Peritonitis	Acute cholecystitis/cholangitis
Severe bleeding disorder	Choledocholithiasis (untreated)
	Gallstone pancreatitis
	Cirrhosis
	Carcinoma/diverticulitis/inflammatory bowel disease
	Inability to tolerate general anesthesia
	Minor bleeding disorder (aspirin, *etc.*)
	Pregnancy
	Obesity

examination, laboratory testing, and ultrasonographic evaluation of the gallbladder. Patients with suggested choledocholithiasis, peptic ulcer disease, or symptomatic gastroesophageal reflux also were referred for either endoscopic or contrast radiographic evaluation of the upper gastrointestinal tract. Additional diagnostic tests in selected patients included computed tomography, oral cholecystography, or biliary scintigraphy.

Three hundred forty-one patients were evaluated on an elective basis and scheduled for surgery. The remaining 34 patients were admitted emergently with a diagnosis of acute cholecystitis (24), gallstone pancreatitis (6), or acute cholangitis (1).

Three hundred seventy-one patients had surgery performed under general anesthesia, and the remaining four had combined regional anesthesia/intravenous sedation.[6] All patients were prepared and draped as for routine open cholecystectomy. A nasogastric (or orogastric) tube was inserted in all patients, except for the four patients who underwent regional anesthesia. A urinary catheter was introduced in all patients except one who had undergone previous major reconstruction of the external urethral meatus. Perioperative antibiotics were administered in all patients.

A four-puncture technique was used in all 375 patients.[2] The positioning of the puncture sites is shown in Figure 1. Five patients underwent a second, simultaneous laparoscopic procedure (tubal ligation, 3 patients; highly selective vagotomy, 1 patient; and a liver biopsy, 1 patient), thereby necessitating placement of an additional trocar. Pneumoperitoneum, using carbon dioxide, was established using standard laparoscopic techniques through either a "closed" or "open" approach. Three hundred forty-eight patients (93%) had pneumoperitoneum established by a closed technique, with insertion of a Verres needle blindly through a supraumbilical incision. Insertion of the umbilical cannula by an open approach[7] was deemed necessary in the remaining 27 patients (7%) because of previous abdominal surgery. Intra-abdominal pressure was maintained between 12 and 15 mm Hg by a high-flow (>6 L/min) insufflator. Further details on the technique of laparoscopy are available in recent publications.[7-9]

Operative visualization was provided by a miniaturized video camera (MP Video, Hopkinton, MA) attached to a 10-mm laparoscope (Cabot Medical, Langhorne, PA) inserted through a 10-mm or 11-mm umbilical cannula (SurgiPort®, United States Surgical Corporation, Norwalk, CT). Retraction of the gallbladder was accomplished by grasping forceps placed through the two lateral 5-mm cannulas (Fig. 2). Exposure of the cystic duct and artery was accomplished with a curved, 10-mm laparoscopic dissector (Maryland Dissector; American Surgical Instruments, Pompano Beach, FL [Fig. 3]). The cystic duct and artery were individually ligated with titanium clips (EndoClip, United States Surgical Corporation) or suture ligature (SurgiTie, United States Surgical Corporation).

Intraoperative cholangiography was performed after identification of the cystic duct. A small incision was made in the cystic duct near its junction with the gallbladder. After this, a flexible catheter was introduced into the cystic duct by means of a specially designed cholangiography clamp (Cabot Medical) (Fig. 4]. The catheter then may be secured in place with the clamp, contrast material injected, and a radiograph obtained in the usual fashion. Once having completed the cholangiogram, the catheter may be removed and the remainder of the cystic duct ligated with surgical clips.

Dissection of the gallbladder from the liver during laparoscopic cholecystectomy was accomplished using monopolar electrocautery (~30 Watts pure coagulation current) in all cases (Fig. 5). After completion of the dissection, the operative field was irrigated with normal saline

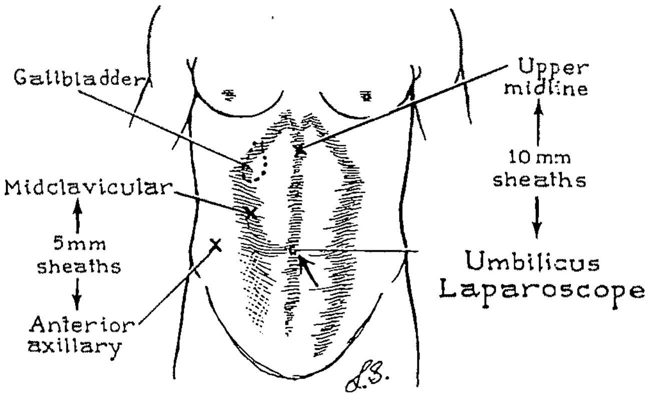

FIG. 1. Recommended sites for trocar insertion during laparoscopic cholecystectomy. The 10/11-mm cannulas are inserted into the umbilical and midepigastric sites. Five-millimeter cannulas are established in the right anterior axillary and right midclavicular lines, 3 to 4 cm below the costal margin. Reproduced with permission from Zucker KA. Laparoscopic guided cholecystectomy with electrocautery dissection. *In* Zucker KA, ed. Surgical Laparoscopy. St. Louis: Quality Medical Publishing, Inc., 1991.

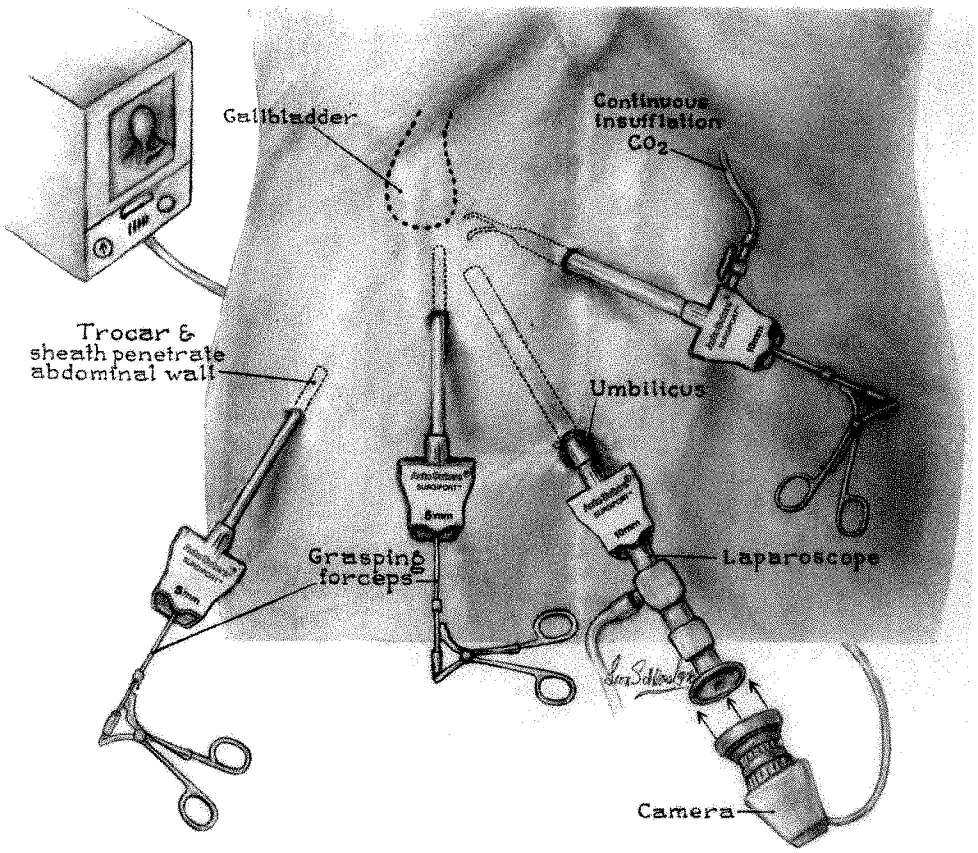
Gallbladder
Continuous insufflation CO2
Trocar & sheath penetrate abdominal wall
Umbilicus
Grasping forceps
Laparoscope
Camera

FIG. 2. Position and function of laparoscopic trocars and instruments during laparoscopic cholecystectomy. The umbilical port allows access for the laparoscope with the attached video camera and also serves as the site of gallbladder extraction at the end of the operation. The midepigastric port functions as the operating cannula. Dissecting instruments, the clip applier, scissors, and suction irrigation devices pass through this site. The two lateral 5-mm cannulas allow access for the grasping forceps. These forceps are used to elevate and retract the gallbladder during dissection of the cystic duct and artery. Reproduced with permission from Zucker KA. Laparoscopic guided cholecystectomy with electrocautery dissection. *In* Zucker KA, ed. Surgical Laparoscopy. St. Louis: Quality Medical Publishing, Inc., 1991.

and hemostasis confirmed by visual inspection. Closed suction drainage was established in five patients, at the discretion of the surgeon. The gallbladder was removed through the umbilical incision by application of an 11-mm penetrating ("claw") forceps to the neck of the gallbladder (Fig. 6). Closure of the umbilical fascial defect was accomplished with a absorbable suture. All four skin incisions were approximated by either skin staples or subcuticular suture.

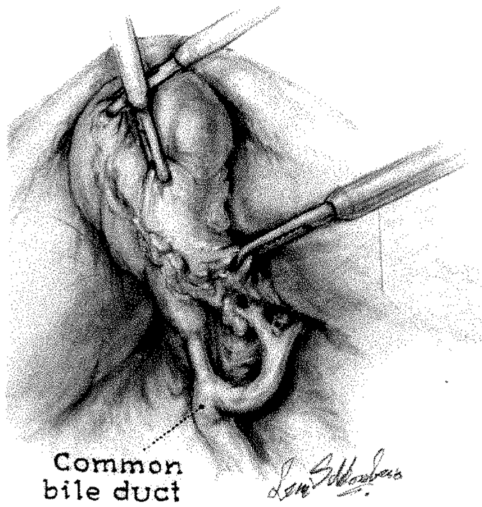

FIG. 3. Exposure of the cystic duct and artery is accomplished by cephalad retraction on the dome of the gallbladder, combined with lateral retraction of the neck of the gallbladder. The cystic duct and artery are identified with the use of an atraumatic curved laparoscopic dissecting instrument. Reproduced with permission from Zucker KA. Laparoscopic guided cholecystectomy with electrocautery dissection. *In* Zucker KA, ed. Surgical Laparoscopy. St. Louis: Quality Medical Publishing, Inc., 1991.

Results

Patient Presentation

Indications for surgery are listed in Table 2. Three hundred forty-one patients (91%) presented on an elective basis, whereas the remaining 34 patients (9%) were admitted for treatment of acute cholecystitis (24 patients), gallstone pancreatitis (9 patients), or cholangitis (1).

Ultrasonography demonstrated cholelithiasis in all but 10 patients. Seven patients were diagnosed as having symptomatic gallbladder disease (chronic cholecystitis) without ultrasonographic evidence of cholelithiasis. The remaining three patients presented with hyperplastic polyps (1), gallbladder dyskinesia (1), and cholangitis (1). The diagnoses in the 10 patients without documented gallbladder calculi were based on strong clinical suspicion, gallbladder wall thickening, abnormal biochemical tests, or abnormal biliary scintigraphy.

Additional diagnostic tests were available in 103 patients, consisting of upper gastrointestinal contrast ra-

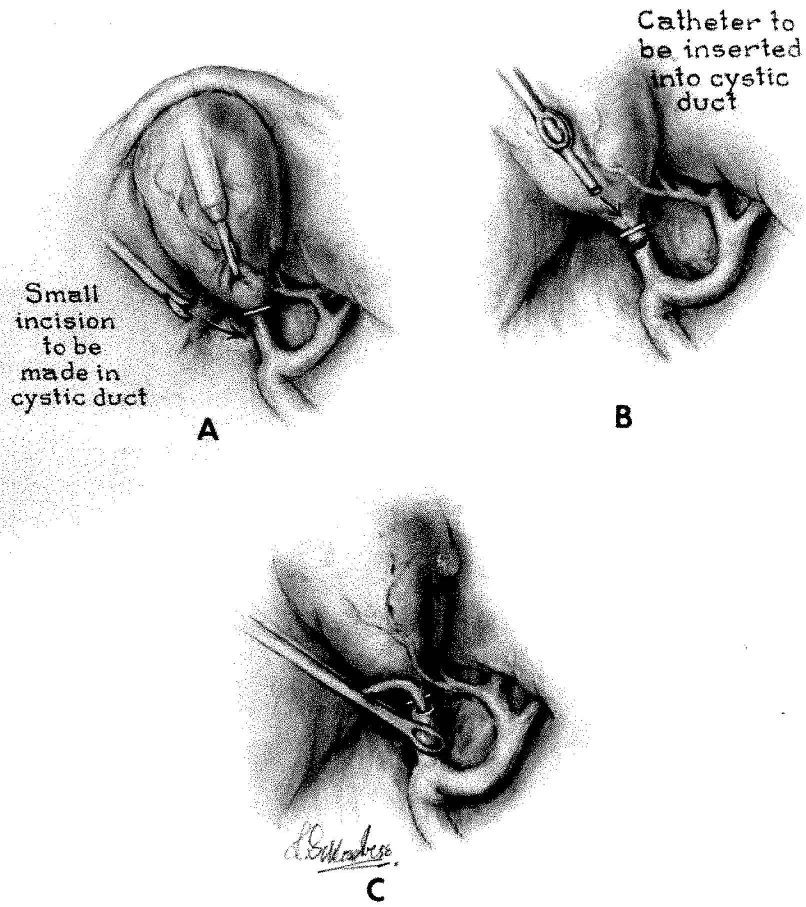
Catheter to be inserted into cystic duct
Small incision to be made in cystic duct
A
B
C

FIG. 4. Technique of laparoscopic cholangiography. (A). A small opening is made in the cystic duct with laparoscopic microscissors. (B). A flexible catheter is inserted through the center of a specially designed cholangiographic clamp and directed toward the cystic duct. (C). Atraumatic forceps located on the end of the instrument are used to secure the catheter within the cystic duct and prevent extravasation of contrast material. Reproduced with permission from Bailey RW, Zucker KA. Laparoscopic cholangiography and management of choledocholithiasis. *In* Zucker KA, ed. Surgical Laparoscopy. St. Louis: Quality Medical Publishing, Inc., 1991.

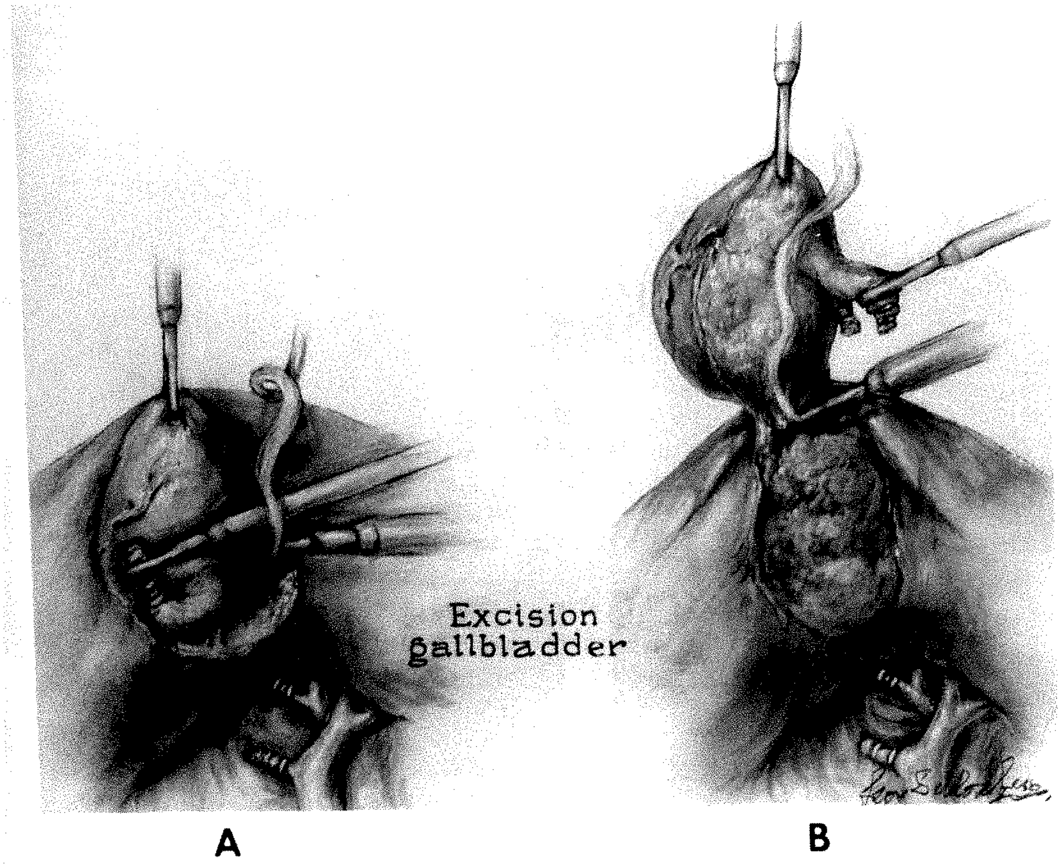

FIG. 5. Electrocautery dissection of the gallbladder. A curved laparoscopic spatula is used to dissect the gallbladder away from its attachments to the liver. Monopolar electrocautery is used to provide adequate tissue separation and hemostasis. The dissection proceeds in an antegrade fashion (from gallbladder neck to dome). The lateral 5-mm forceps are used to provide exposure of the gallbladder, thereby facilitating its removal. Reproduced with permission from Zucker KA. Laparoscopic guided cholecystectomy with electrocautery dissection. *In* Zucker KA, ed. Surgical Laparoscopy. St. Louis: Quality Medical Publishing, Inc., 1991.

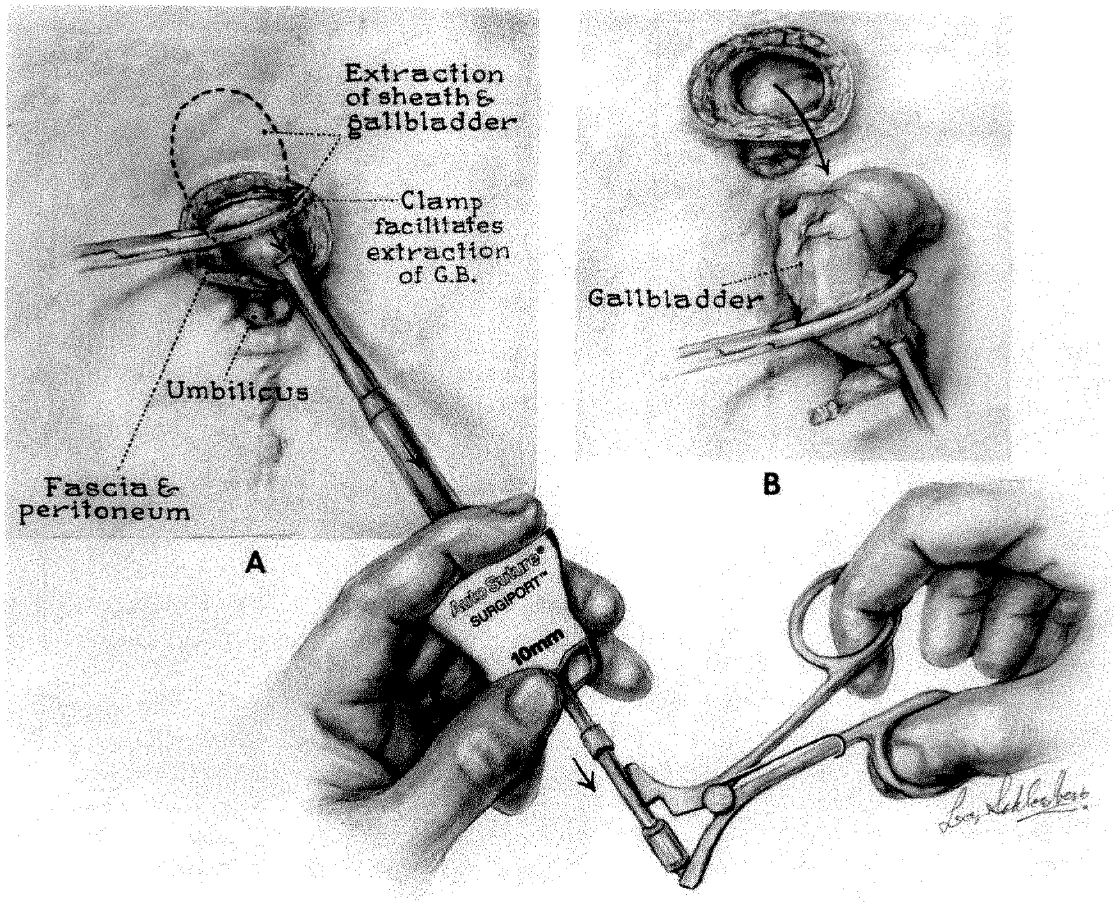
Extraction of sheath & gallbladder
Clamp facilitates extraction of G.B.
Umbilicus
Fascia & peritoneum
A
Gallbladder
B
Auto Suture®
SURGIPORT™
10mm

FIG. 6. The gallbladder is removed through the umbilical incision. To accomplish this, the laparoscope with attached video camera is first relocated to the upper midline portal. Large grasping forceps are inserted through the umbilical cannula and placed on the neck of the gallbladder. The forceps are then withdrawn into the umbilical sheath, and both the sheath and the forceps are simultaneously withdrawn through the umbilical fascial opening. The neck of the gallbladder is secured at the skin with a curved clamp, and the remainder of the gallbladder is delivered through the umbilicus. Reproduced with permission from Zucker KA. Laparoscopic guided cholecystectomy with electrocautery dissection. *In* Zucker KA, ed. Surgical Laparoscopy. St. Louis: Quality Medical Publishing, Inc., 1991.

diography (48) or endoscopy (39), biliary scintigraphy (43), computed tomography (33), oral cholecystography (10), barium enema(6), colonoscopy (4), or intravenous pyelography (3).

Twenty-four patients (6%) presented with acute cholecystitis. A partial report on these patients has been recently published.[10] The diagnosis was determined by clinical criteria, laboratory data, ultrasonography, or nuclear medicine scan. All 24 patients had right upper quadrant pain with localized tenderness. Nineteen patients (79%) were febrile to 100 F or greater on admission. An elevated white blood cell count ($>10,000/m^3$) was present in 18 patients (75%). Sonography showed cholelithiasis in all 24 patients and findings consistent with acute cholecystitis (wall thickening, pericholic fluid, positive sonographic Murphy's sign) in 21 patients. Biliary scans were obtained in 12 patients and showed cystic duct obstruction in 11 and delayed visualization in one patient. Patients admitted with a diagnosis of acute cholecystitis were treated with intravenous hydration, systemic antibiotics, and urgent surgery within 72 hours of presentation. The clinical diagnosis of acute cholecystitis was confirmed by gross and histologic evaluation in all 24 patients. A diagnosis of gallstone pancreatitis or cholangitis was made by standard clinical, laboratory, and radiographic criteria. All patients with gallstone pancreatitis or cholangitis underwent laparoscopic cholecystectomy or preoperative ERCP within 12 to 72 hours of admission. The one patient with chol-

TABLE 2. *Indications for Surgery*

Indication	No. of patients	%
Biliary colic	332	89
Acute cholecystitis	24	6
Gallstone pancreatitis	9	2
Chronic cholecystitis	7	2
Cholangitis	1	0.3
Gallbladder polyps	1	0.3
Gallbladder dyskinesia	1	0.3

angitis underwent urgent endoscopic biliary drainage, followed by successful laparoscopic cholecvstectomy 4 days later.

Prevalence of Relative Contraindications

Of 375 patients 160 (43%) presented with a history of previous abdominal surgery (Table 3). Thirty-one of these patients had a previous laparoscopic procedure performed through an umbilical incision.

One hundred twenty-four patients presented with significant comorbid disease (Table 3). The most important findings included 64 patients with hypertension, 34 with documented coronary artery disease (including 19 patients with previous myocardial infarction), 13 patients with chronic pulmonary disease, and 13 patients with diabetes mellitus. A history of peptic ulcer disease and gastroesophageal reflux was elicited in 17 and 6 patients, respectively.

Conversion Rate

Laparoscopic cholecystectomy was successful in 355 of 375 attempts (95%), for an overall conversion rate of 5%. The remaining 20 patients were converted to laparotomy and open cholecystectomy. Elective cases were successful in 328 of 341 patients, for a conversion rate of 4%, whereas conversion to open cholecystectomy was required in 7 of 24 patients (30%) presenting with acute cholecystitis. All attempts at laparoscopic cholecystectomy in patients admitted with gallstone pancreatitis (9 patients) or cholangitis (1 patient) were successful.

Immediate conversion to laparotomy was performed at the discretion of the surgeon and was due to unusual intraoperative findings in 17 patients (4.5%) or to intraoperative complications in three patients (0.8%). Laparotomy during the immediate postoperative period was required in an additional three patients (0.8%) because of a cystic duct injury, delayed cystic duct necrosis at 10 days after surgery, and a single common hepatic duct injury. Indications for conversion are listed in Table 4. Perioperative complications leading to immediate or delayed laparotomy include injury to the cystic duct (3 patients), common bile duct injury (1 patient), bile leakage from a small accessory bile duct (1 patient), and delayed necrosis of the cystic duct stump (1 patient).

TABLE 3. *Prior Surgery and Comorbid Illness*

Previous Abdominal Surgery 160 patients (43%)	No.	Comorbid Disease 124 patients (33%)	No.
Confined to lower abdomen	144	Hypertension	64
		Coronary artery disease	34
Confined to upper abdomen	5	Myocardial infarction	19
Upper and lower abdomen	11	Chronic pulmonary disease	13
Previous laparoscopy	31	Diabetes mellitus	13
		End stage renal disease	4
		HIV+ infection	2

TABLE 4. *Conversion Data**

Due to Intraoperative Findings 17/375 (4.5%)	Due to Complications 6/375 (1.6%)
Chronic inflammation (7)	Cystic duct injury (3)
Acute inflammation (6)	Common duct injury (1)
Empyema/abscess (1)	Accessory bile leakage (1)
Pancreatic mass (1)	Cystic duct necrosis (1)
Suspected liver metastases (1)	
Common bile duct stones/failed ERCP (1)	

* Immediate conversion rate: 20/375 cases (5.3%); reoperation rate (delayed conversion): 3/375 cases (0.8%); overall exploration rate: 23/375 cases (6.1%).

ERCP, endoscopic retrograde cholangiopancreatography.

Morbidity

Overall morbidity was 3.5% and can be divided into major complications in two patients (0.6%) and minor complications in 11 patients (2.9%; Table 5). Major morbidity included a single common hepatic duct injury, as described in Figure 7, and delayed necrosis of the cystic duct stump at 10 days. The latter patient had undergone uncomplicated laparoscopic cholecystectomy and was discharged on postoperative day 1. Follow-up evaluation at 8 days after surgery was unremarkable; however on postoperative day 10 the patient developed spontaneous bile peritonitis. Exploration (at an outside institution) showed necrosis of the cystic duct stump. The previously placed clips were found to be intact, and there was no evidence of direct injury to the cystic duct. Both patients who required laparotomy for these perioperative complications are alive and well at 16-month follow-up.

Major reconstruction of the extrahepatic biliary system was required in only two patients, one of whom represents the single common hepatic duct injury. The other patient presented electively for gallbladder surgery and was found at surgery to have a choledochocolonic fistula. The bi-

TABLE 5. *Morbidity and Mortality*

Major Complication	No. (%)	Minor Complication	No. (%)
Common bile duct injury	1 (0.3)	Cystic duct injury	3 (0.9)
Cystic duct necrosis	1 (0.3)	Decreased hematocrit	2 (0.6)
		Urinary retention	2 (0.6)
		Rectus hematoma	1 (0.3)
		Deep vein thrombosis	1 (0.3)
		Mild aspiration	1 (0.3)
		Accessory duct leak	1 (0.3)
Total major	2 (0.6)	Total minor	11 (2.9)
Overall morbidity			13 (3.5)
Overall mortality (Myocardial infarction on postoperative day 3)			1 (0.3)

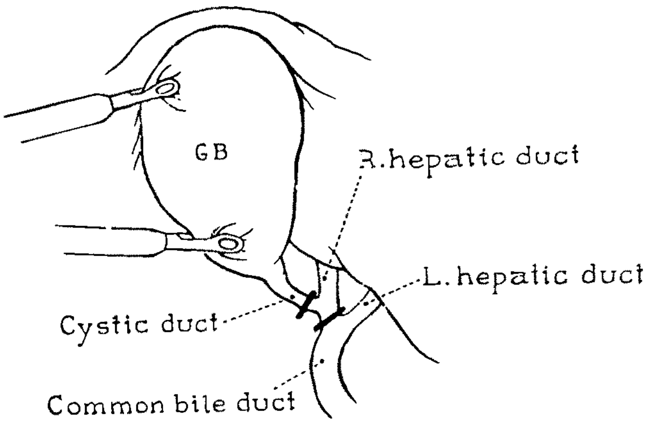

FIG. 7. Illustration of common hepatic duct injury. Failure to recognize an aberrant entrance of the cystic duct into the right hepatic duct and incomplete dissection of the entire cystic duct most likely lead to the injury shown here. Clips were correctly placed on the distal cystic duct, near its junction with the gallbladder; however, clips were incorrectly placed across the proximal end of the cystic duct, leading to the injury shown. The injury required reconstruction with a Roux-en-Y limb of jejunum. Reproduced with permission from Zucker KA. Laparoscopic guided cholecystectomy with electrocautery dissection. *In* Zucker KA, ed. Surgical Laparoscopy. St. Louis: Quality Medical Publishing, Inc., 1991.

furcation of the right and left hepatic ducts was completely obliterated by the inflammatory process, thereby requiring biliary reconstruction with a Roux-en-Y limb of jejunum and colonic resection.

Minor complications included three cystic duct injuries, a decrease in the postoperative hematocrit in two patients (one requiring transfusion, none requiring re-exploration), transient (<12 hours) urinary retention in two patients, and one case each of an accessory bile duct leak, a rectus sheath hematoma (resolved spontaneously), a lower extremity deep vein thrombosis (treated with standard anticoagulation therapy), and a mild episode of aspiration during the induction of anesthesia. (This patient developed no significant clinical findings during the immediate postoperative period.) A small stitch abscess was observed in one patient and resolved after removal of the suture.

There were no postoperative wound infections, intra-abdominal sepsis, major pulmonary complications, postoperative pancreatitis, major vascular injuries, or visceral perforation. Among the 23 patients undergoing conversion to laparotomy, there were two minor complications. One patient developed a wound infection, and the other developed an incisional hernia 6 months after surgery.

Death

A single perioperative death occurred, for an overall mortality rate of 0.3%. This death occurred in a 75-year-old man with a documented myocardial infarction 4 years before his elective gallbladder surgery. Preoperative evaluation also included a stress thallium scan, an echocardiogram, and cardiac catheterization. The patient was not believed to be a candidate for coronary artery bypass grafting or balloon angioplasty. The patient underwent uncomplicated laparoscopic cholecystectomy but developed transient urinary retention on postoperative day 1. The catheter was removed on postoperative day 2, and the patient was able to void spontaneously. Postoperative electrocardiographic evaluation and creatine phosphokinase-MB band analysis were unremarkable. While awaiting discharge on postoperative day 3, however, the patient suffered a ventricular arrhythmia and subsequent massive reinfarction of his myocardium. The patient died shortly thereafter. No deaths occurred in the 23 patients converted to open cholecystectomy.

Length of Hospitalization and Recovery Periods

Details concerning the time of discharge and the time to return to normal activity for all 375 patients are listed in Table 6. The mean hospital stay was 1.3 ± 0.1 days. Among the 355 patients having successful laparoscopic cholecystectomy, 319 patients (90%) were discharged within 24 hours of surgery. One patient underwent successful laparoscopic cholecystectomy but was found to have previously unsuspected common bile duct stones by intraoperative cholangiography. Postoperative ERCP showed two calculi in the common bile duct as well as a 1.5-cm stricture of the distal common bile duct. This patient remained in the hospital for an additional 12 days, during which time the stones were cleared from the ductal system and the stricture was adequately treated by both radiographic and endoscopic manipulation. The patient was discharged on postoperative day 14, tolerating a regular diet and having resumed full, normal activity.

Twenty-three patients were converted to laparotomy, as previously described. None of these 23 patients were discharged before postoperative day 4. The time to dis-

TABLE 6. *Hospitalization and Recovery Data*

Laparoscopic Cholecystectomy n = 355		Open Cholecystectomy n = 20	
Time of discharge		Time of discharge	
Same day	17 (5%)	POD 0–3	0 (0%)
POD 1	302 (85%)	POD 4	6 (30%)
POD 2	21 (6%)	POD 5	7 (35%)
POD 3	7 (2%)	POD 6–7	5 (25%)
POD 4–7	5 (1%)	POD 11–21	2 (10%)
POD 8–14	3 (1%)		
Mean hospital stay (days)	1.3 ± 0.1		4.8 ± 0.9
Return to normal activity (n = 266)		Return to normal activity	
5 days	35 (13%)	3 weeks	1 (5%)
7 days	208 (78%)	4 weeks	6 (30%)
14 days	14 (5%)	5 weeks	5 (25%)
30 days	9 (3%)	6 weeks	5 (25%)
		8 weeks	3 (15%)

POD, postoperative day.

charge in these 23 patients is also presented in Table 3. The mean hospital stay of patients undergoing open cholecystectomy was 4.8 ± 0.9 days.

The duration of postoperative pain was estimated in 315 of 355 patients undergoing laparoscopic cholecystectomy. The presence of shoulder, umbilical, and generalized abdominal pain were analyzed. Two hundred seventy-nine patients did not experience shoulder pain, and any discomfort that was present in the remaining 36 patients had disappeared completely within 24 hours. Similarly 251 of 315 patients reported no umbilical pain and, by 24 hours after surgery, the umbilical discomfort had resolved in all but 10 of the remaining patients. Generalized abdominal discomfort was not uncommon after laparoscopic cholecystectomy but was still absent in 107 of 315 patients. By 24 hours after surgery this pain had resolved in 224 of 315 patients and, by 48 hours, had completely disappeared in 288 of the 315 patients evaluated.

Information on postoperative recovery times was available in 266 of 355 patients undergoing laparoscopic cholecystectomy. The average time for return to normal activity after laparoscopic cholecystectomy was 7 days (Table 6). Of 266 patients 243 (91%) had returned to full, normal activity by 7 days after successful laparoscopic surgery. The average return to normal activity in the 23 patients undergoing laparotomy was 5 weeks (Table 6).

Cholangiography

Operative cholangiography was attempted in 156 of 355 successful laparoscopic procedures (44%), at the discretion of the surgeon. Although selective cholangiography was advocated during the initial phase of this study, most of the authors now perform routine cholangiography. Laparoscopic cholangiography was successful in 141 of 156 attempts (90%). Evidence of choledocholithiasis was documented in five patients, for a 4% incidence of positive studies among those patients undergoing cholangiography. These five patients were treated by postoperative ERCP in four cases and concurrent open common bile duct exploration in one. This last patient had undergone a failed preoperative attempt at ERCP, which thereby mandated open exploration once calculi were documented on intraoperative cholangiography.

TABLE 7. *Cholangiography and Laparoscopic Cholecystectomy*

Cholangiography Data	Patient No.	%
Attempts	156/355	44
Studies performed	141/355	40
Successful attempts	141/156	90
Positive study	5/141	4
Treatment		
ERCP	4/5	
CBDE	1/5	
Not attempted	214/355	60
Retained stones	2/355	0.6

ERCP, endoscopic retrograde cholangiopancreatography. CBDE, common bile duct exploration.

Two retained stones have been detected during follow-up in 214 patients not having intraoperative cholangiography, for an incidence of unrecognized retained stones of 0.9%. A total of seven patients in the entire series (355 successful laparoscopic cases) were found to have previously unsuspected common bile duct stones (2%). Only two of these patients (0.5%), however, went unrecognized during surgery. Details of the results from cholangiography are given in Table 7.

Operative Time

The mean operative time for 355 laparoscopic cases was 1.7 ± 0.1 hours (range, 0.6–3.75 hours). For elective cholecystectomy the mean operative time was also 1.7 ± 0.1 hours (range, 0.6–3.75), whereas for acute cholecystitis it was 2.0 ± 0.1 hours (range, 0.9–3.5 hours). Operative time varied considerably, depending on whether or not cholangiography was performed, whether a resident performed the operation, and also on the experience of the surgeon.

Resident Training

Resident training was begun early in our experience. Patients with acute cholecystitis or cholangitis were automatically excluded from resident teaching. All cases converted to laparotomy were not included in the analysis of data concerning resident training. Three hundred forty-one cases therefore were available as potential resident teaching cases. Senior residents successfully completed the operation in the role of surgeon in 235 (69%) of these patients. The remaining 106 cases (31%) were performed by the attending surgeon, with the senior resident serving as first assistant. The mean operative time for a resident-completed laparoscopic case was 1.7 ± 0.1 hours (range, 0.8–3.75).

Discussion

At our institution laparoscopic cholecystectomy has replaced open cholecystectomy as the procedure of choice for the treatment of symptomatic gallbladder disease. Randomization of patients between these two surgical options has not been possible because of the early recognition of the potential advantages of a laparoscopic approach. In a similar fashion, this procedure has been adopted so rapidly in the United States and Europe that large randomized, controlled studies have not been forthcoming.

Many coexisting conditions such as acute cholecystitis, cholangitis, previous abdominal surgery, or pregnancy initially were believed to represent absolute contraindications to laparoscopic cholecystectomy. With increased experience many of these factors are now considered to be only relative contraindications. Currently it is our view that patients presenting with evidence of sepsis or generalized peritonitis represent an absolute contraindication to laparoscopic cholecystectomy and should undergo emergent surgical treatment by laparotomy. Although laparoscopic cholecystectomy might be possible in these situations, operative time can be expected to be considerably increased (2–3 hours). Such delays in patients who are hemodynamically unstable probably would serve only to increase operative morbidity and mortality. The presence of a severe bleeding disorder also constitutes another absolute contraindication. Control of significant operative hemorrhage is more difficult under laparoscopic guidance because the placement of surgical packs or direct manual compression of bleeding sites is not easily accomplished under laparoscopic guidance.

During the study period, a primary laparoscopic approach to symptomatic gallbladder disease was undertaken in 375 of 403 patients. Therefore over 93% of patients with gallbladder disease were candidates for laparoscopic cholecystectomy. If one were to exclude those cases that were not attempted because of surgeon inexperience, the percentage of patients undergoing primary open cholecystectomy would be even lower. Among 375 attempts 355 were completed successfully. A substantial number of these patients presented with acute cholecystitis, gallstone pancreatitis, previous abdominal surgery, and other relative contraindications. Laparoscopic cholecystectomy was both indicated and successful in more than 90% of patients and thereby differs from other nonsurgical alternatives to cholecystectomy such as oral dissolution or lithotripsy treatment. During the initial phases of this series, several patients with relative contraindications were not offered laparoscopic cholecystectomy. With increased experience, however, we have found that even patients who present with relative contraindications can still undergo an initial laparoscopic approach to the gallbladder.

Initial reports from several centers have suggested the potential superiority of laparoscopic cholecystectomy over an open approach.[1,3] The conclusions of these earlier studies are further supported by the current series. Ninety per cent of patients having successful laparoscopic cholecystectomy are discharged from the hospital within 24 hours, and over 91% have returned to full, normal activity by 1 week. This is clearly in distinct contrast to the large experience with standard open cholecystectomy,[11] and even with the so-called "mini-laparotomy cholecystectomy."[2] Additional benefits include decreased postoperative discomfort as evidenced by subjective patient evaluation and early return to normal activity. Avoidance of a large subcostal or midline incision has obvious appeal to many patients and has contributed to the popularity of this new procedure. One other striking advantage is the potential savings in health care costs. Although data are still somewhat limited, substantial savings are expected. When considering that more than 500,000 cholecystectomies are performed every year in the United States,[12] the savings to the country as whole will be significant if based solely on an average return to work of 7 days as compared with 4 to 6 weeks after open cholecystectomy.

Despite these potential advantages of laparoscopic cholecystectomy, concern over the safety of laparoscopic cholecystectomy has been raised.[13–15] The current study provides further information regarding the overall morbidity and mortality associated with laparoscopic cholecystectomy. Certainly more centers will have to report on their experience before the question of safety can be completely answered.

It would appear from the current data that laparoscopic cholecystectomy can be performed as quickly, as efficiently, and with the same morbidity and mortality as with open cholecystectomy. A single common bile duct injury in 375 cases is within the acceptable range for this complication.[16] This injury occurred during the very early phases of our experience and may illustrate the important role for adequate training and credentialling. A mortality rate of 0.3% also agrees favorably with data reported from large series on open cholecystectomy.[17] Furthermore we have not witnessed an untoward number of additional complications such as bowel injury that might indicate an increased morbidity associated with laparoscopic surgery. On the contrary among 355 patients undergoing successful laparoscopic cholecystectomy, there were no postoperative wound infections, intra-abdominal sepsis, major pulmonary complications, postoperative pancreatitis, major vascular injury, or visceral perforation. This is less than would be expected based on the results of previous reports analyzing large numbers patients after open cholecystectomy.[11,17]

Many surgeons have been reluctant to use monopolar cautery during laparoscopic surgery. This has been due largely to fear of inadvertent bowel injury due to "arcing" from the electrocautery probe. In fact earlier reports in the gynecologic literature have commented on the potential hazards of monopolar electrosurgery during laparoscopy. This theory has been challenged in a recent report by Soderstrom and Levy,[18] however. This article provides evidence that many of the injuries originally ascribed to monopolar current were most likely the result of either surgeon error (traumatic injury) or faulty and unstable equipment. Recent experimental evidence also supports this contention.[19] In our series of 375 cases, we have not experienced a single injury related to the use of electrocautery. We have found electrocautery to be safe, effective, and relatively inexpensive compared with other modalities. Furthermore we have not witnessed any significant clinical disadvantage to patient recovery after electrocautery dissection of the gallbladder.

Laparoscopic cholecystectomy can be performed with the same efficacy and safety as open cholecystectomy. In the authors' opinion, the striking advantages of laparoscopic cholecystectomy have rendered the performance of a primary "open" cholecystectomy on an elective basis unwarranted in most circumstances. Among the published reports, there does not appear to be an overwhelming incidence of perioperative complications. Because laparoscopic surgery offers such significant advantages and is clearly "here to stay,"[13] perhaps our current efforts should be directed toward identifying current limitations, eliminating sources of operative error, and improving existing technology (*e.g.*, three-dimensional video system). Only with such endeavors and the assurance of proper training[20] can future reductions in perioperative morbidity rates be expected.

Acknowledgements

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References

1. Zucker KA, Bailey RW, Gadacz TR, Imbembo AL. Laparoscopic guided cholecystectomy. Am J Surg 1991; 161:36–44.
2. Reddick EJ, Olsen DO. Laparoscopic laser cholecystectomy: a comparison with mini-lap cholecystectomy. Surg Endosc 1989; 3:44–48.
3. Dubois F, Icard P, Berthelot G, Levard H. Coelioscopic cholecystectomy: a preliminary report of 36 cases. Ann Surg 1990; 211: 60–62.
4. Salky BA, Bauer JJ, Kreel I, et al. Laparoscopic cholecystectomy: an initial report. Gastrointest Endosc 1991; 37:1–4.
5. Peters JH, Ellison EC, Ines JT, et al. Safety and efficacy of laparoscopic cholecystectomy: a prospective analysis of 100 initial patients. Ann Surg 1991; 213:3–12.
6. Hasnain JU, Matjasko MJ. Practical anesthesia for laparoscopic procedures. *In* Zucker KA, Bailey RW, Reddick EJ, eds. St Louis: Quality Medical Publishing, 1991, pp 77–86.
7. Fitzgibbons RJ Jr, Salerno GM, Filipi CJ. Open laparoscopy. *In* Zucker KA, Bailey RW, Reddick EJ, eds. Surgical Laparoscopy. St Louis: Quality Medical Publishing, 1991, pp 87–97.
8. Flowers JL, Zucker KA, Bailey RW. Laparoscopic cholecystectomy. Surg Round 1991; 14:271–282.
9. Zucker KA. Laparoscopic guided cholecystectomy with electrocautery dissection. *In* Zucker KA, Bailey RW, Reddick EJ, eds. Surgical Laparoscopy. St Louis: Quality Medical Publishing, 1991, pp 143–182.
10. Flowers JL, Bailey RW, Scovill WA, Zucker KA. The Baltimore experience with laparoscopic management of acute cholecystitis. Am J Surg 1991; 161:388–392.
11. Ganey JB, Johnson PA Jr, Prillaman PE, McSwain GR. Cholecystectomy: clinical experience with a large series. Am J Surg 1986; 151:352–357.
12. National Inpatient Profile. Ann Arbor: Healthcare Knowledge Systems, 1989, pp 360–363.
13. Cameron JL, Gadacz TR. Laparoscopic cholecystectomy. Ann Surg 1991; 213:1–2 [Editorial].
14. Cuschieri A, Berci G, McSherry CR. Laparoscopic cholecystectomy. Am J Surg 1990; 159:273 [Editorial].
15. Wolfe BM, Gardiner B, Frey CF. Laparoscopic cholecystectomy: a remarkable development. JAMA 1991; 265:1573–1574.
16. Henry ML, Carey LC. Complications of cholecystectomy. Surg Clin North Am 1983; 63:1191–1204.
17. McSherry CK. Cholecystectomy: the gold standard. Am J Surg 1989; 158:174–178.
18. Soderstrom RM, Levy BS. Bowel injuries during laparoscopy: causes and medicolegal questions. Contemp Obstet Gynecol 1986; 27: 41–45.
19. Soper NJ, Barteau JA, Clayman RV, Becich MJ. Safety and efficacy of laparoscopic cholecystectomy using monopolar electrocautery in the porcine model. Surg Lapar Endosc 1991; 1:17–22.
20. Bailey RW, Imbembo AL, Zucker KA. Establishment of a laparoscopic cholecystectomy training program. Am Surg 1991; 57: 231–236.

DISCUSSION

DR. C. MCSHERRY (New York, New York): I compliment Dr. Bailey and his associates in Baltimore for their excellent results, and I think there are enough people in this audience and in this Society who have comparable experience that also would indicate that this procedure is safe, and it certainly is here to stay, and I think the interest in coming years will be in what other applications this laparoscopic approach can be put to.

I think, unfortunately, that the Bailey experience is not the same throughout the country. We still see a fair number of common duct injuries, and septic complications secondary to bile leaks, but that should not detract from the importance of this procedure. It can be done safely, and it can be done with great advantage to the patients.

Perhaps Dr. Bailey might like to comment and give us his thoughts on measures we can take to improve the overall safety of this procedure throughout the surgical community. Thank you.

DR. CONDON (Milwaukee, Minnesota): Some of us, perhaps all of us, have recently received from some organization in southern California a not-very-subtle mailing piece outlining medical malpractice liability with use of the electrocautery for a laparoscopic cholecystectomy and advising us, undoubtedly on behalf of their sponsors, that we had better use a laser.

I wonder if Dr. Bailey would comment on this issue.

DR. PETER DECKERS (Farmington, Connecticut): Do the residents begin learning this technique in the operating room assisting on patients, or does this group first take their residents to the animal laboratory and teach the technique there?

DR. S. STRASBERG (Toronto, Canada): Dr. Bailey, have you been able to identify criteria, in those patients who were converted to an open procedure, that might help us identify those patients before the surgery?

My comment is on the use of the words "successfully completed." I would suggest that we try very hard not to establish a bias in surgeons' minds that converting this procedure to an open procedure is "unsuccessful." I think that some of the complications of this procedure might occur as a result of trying too hard to complete the procedure by the laparoscopic method.

DR. J. ALDRETE (Birmingham, Alabama): I wish to congratulate Dr. Bailey and his colleagues for their very good results. I would like to ask them a question, and that is, why do you think this operation, purely from the physiologic point of view, has the great advantage that the patients can leave the hospital the same day of the operation, or the following day, which seldom occurs after open cholecystectomy? After watching my younger colleagues do many of these procedures with a degree of success that parallels that presented by Dr. Bailey, my own hypothesis is that these patients can leave the hospital early and have really minimal discomfort because they never develop an adynamic ileus.

This is somewhat surprising because often during the process of laparoscopic cholecystectomy, bile and even a few gallstones are spilled into the abdominal cavity, which for many years we thought was terrible. Despite this occurrence, most patients after laparoscopic cholecystectomy can leave the hospital the same day of operation, or the following morning. In contrast most patients who undergo open cholecystectomy, no matter how small their incision, still have problems with intestinal peristalsis, which keeps them in the hospital longer.

DR. A. J. WALT (Detroit, Michigan): Using this procedure will you routinely do intraoperative cholangiography, and if you see stones, will you go after them at that same session?

Have you removed stones laparoscopically from the common duct? Lastly do you have a plan formulated for what you will do in patients with acute biliary pancreatitis who have laparoscopic cholecystectomy?

DR. ROBERT W. BAILEY (Closing discussion): Dr. McSherry, you have stressed the importance of training. I believe that training is a critical issue. We have recently presented a paper on this subject at the S.A.G.E.S. conference in Atlanta.

I think, as you said, it is clear that laparoscopic cholecystectomy is here to stay. Emphasis has to be directed toward finding ways of decreasing the operative morbidity and mortality, especially during the early learning phases of the surgeon's experience.

I recently returned from a trip to Japan, where they tell me they have the technology to provide us with a three-dimensional image through the laparoscope. Certainly technology like this will have a dramatic impact on the future of laparoscopic surgery.

One of the major difficulties with laparoscopic surgery at present is the loss of depth perception inherent to working off a two-dimensional video monitor. They now have flexible laparoscopes, which will allow the surgeon to have a deflectable tip inside the abdomen to visualize structures from different angles. So this also will be important in the future.

Dr. Condon, you brought up the issue of electrocautery. As mentioned in our discussion, we have used exclusively electrocautery in all 375 cases. We have not had any injuries whatsoever related to the use of electrocautery. There has been some recent concern, mostly in the gynecologic literature, about the dangers of electrocautery. I would, however, point out that in a recent publication on this issue, many of these cases of presumed electrocautery bowel injuries were reanalyzed. They probably were not related to direct cautery injury from arcing or gapping of the probes, but more likely were due to surgeon error or faulty insulation of the instruments.

In addition there is some recent experimental evidence in animals with the use of electrocautery and, again, no evidence of danger was reported with the use of electrocautery.

With regard to resident training, we have established a formal study program. We try to get as many of the residents as possible over to the animal laboratory ahead of time. Unfortunately because of scheduling constraints that is not always possible. Fortunately with a residency program, you have the luxury of spending time with each individual resident. Their initial exposure to the operation is begun with review of surgical atlases and videotapes and other didactic materials.

From there they proceed into the operating room, usually in the role of the camera assistant, so that they can learn the operative protocol, and then to the role of first assistant, and finally they perform the procedure in the role of surgeon. An experienced laparoscopic surgeon can adequately direct the course of the operation and, as with open surgery, can take a resident through a case, so to speak.

We have not been able to identify any criteria for conversion ahead of time. The only preoperative factor that is clearly related to conversion is the presence of acute cholecystitis. Certainly acute cholecystitis is something that can be identified on admission. Our conversion rate for acute cholecystitis is approximately 30%, and that has remained constant through our series of 24 patients.

What is most surprising is the number of patients presenting electively that end up having very severe, chronic inflammation that was not detected on any of the preoperative studies. So aside from the presence of acute cholecystitis, we do not have any firm criteria at this time for determining preoperatively which patients will go on to conversion.

Dr. Strasberg, I agree with you with that the use of the term "unsuccessful attempts" may not be entirely appropriate. We do not view conversion to laparotomy as a complication, rather as a viable option that every surgeon has in the appropriate treatment of gallstone disease. We did not want to be accused of any bias during our initial presentation, however, and took the worst viewpoint and listed all conversions as being unsuccessful attempts. Certainly in the future we may change this outlook.

Dr. Aldrete commented on the physiologic basis for the advantages of laparoscopic cholecystectomy. Clearly there are two things that are different with laparoscopic removal of the gallbladder as compared with open cholecystectomy. One is the avoidance of a fascial incision, and second is the manipulation of the abdominal viscera. Exactly how much of each of these two components contribute to this, I do not have any data for you at this point. Certainly avoidance of a large fascial incision and manipulation of the bowel has a significant impact on patient recovery.

As far as spillage of bile within the abdomen, that is something that does occur early on in one's experience. As one gains more experience, however, bile spillage is more of an unusual occurrence.

Regarding the management of biliary tract, Dr. Walt, the subject of the management of common bile duct stones during laparoscopic surgery is an extremely controversial one and accounts for extensive further discussion.

Our current management of common bile duct stones detected on operative cholangiography is to proceed with laparoscopic choledochoscopy. Attempts are made to extract stones with basketing forceps, which we have completed successfully.

After that there are two options, to simply complete the laparoscopic cholecystectomy and refer the patient for postoperative ERCP, and especially if you have confidence in your endoscopist, that is a very viable option. The other option, which we do not recommend routinely, is to immediately convert to an open common bile duct exploration.

There are new reports just recently published on laparoscopic choledochotomy and laparoscopic common bile duct exploration. Certainly it would seem intuitive that once we have gained the necessary technical skills, direct choledochotomy with common bile duct exploration, placement of a T-tube, and suturing of the T-tube within the common bile duct may become commonplace in the future.

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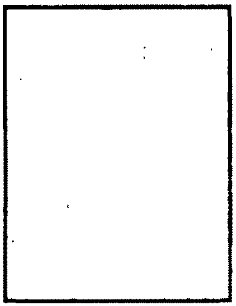
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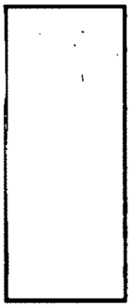
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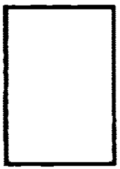
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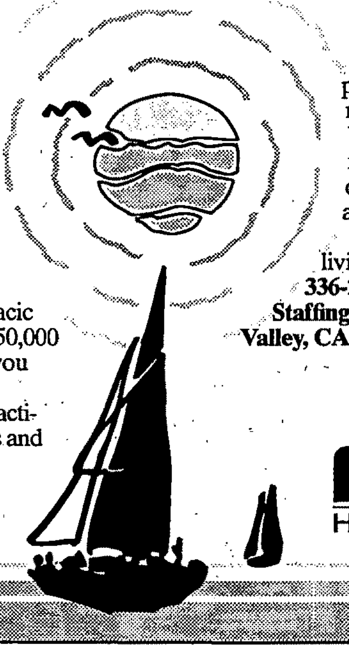

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Implications of Malignant-Cell DNA Content for Treatment of Patients With Pancreatic Cancer

Ductal adenocarcinoma of the pancreas kills almost all patients who contract it, the overall 5-year survival being about 3%,[1] with a median survival of only about 6 months.[2] Only those in whom the cancer arises in the head of the gland have a chance to be cured, and the only known curative treatment is complete surgical removal by pancreatoduodenectomy. The dismal statistics and the previously formidable complication and mortality rate of the Whipple operation have led some physicians of pessimistic bent to suggest that attempted resection is not worthwhile and to advocate palliative bypass or stenting only.[3,4] The circumstances are clearly changing, however. Institutions around the world with larger experiences in pancreatic resection are reporting dramatically reduced mortality rates for pancreatoduodenectomy. Compared with figures in excess of 20% in the 1970s, current postoperative mortality should be well under 5%.[5-8] Actuarial 5-year survivals of *resected* patients has also greatly improved for unknown reasons. For example it currently stands at 17% at our hospital, 19% at Johns Hopkins,[9] and 24% at Mannheim.[7]

Coupled with the improved outcomes has been a movement toward better selection of patients for attempted resection. With the ability to perform biopsies percutaneously and to palliate jaundice effectively by nonoperative stenting,[10] there is no longer a need to operate on every patient, and only duodenal obstruction remains a relatively absolute indication for surgery. In this setting it is of manifest importance to search preoperatively for evidence of incurability. Larger metastases are detected by computed tomography (CT) and ultrasound. Smaller liver, peritoneal, and omental metastases can be seen and biopsy done by laparoscopy.[11,12] Encasement of the major mesenteric vessels, especially the portal-mesenteric vein, is demonstrable by CT or angiography.[12] Evidence of intraperitoneal dissemination by cytologic examination of peritoneal washings has recently been suggested as another parameter, perhaps indicating the probable futility of resection.[13]

Allison and his colleagues from Johns Hopkins[14] are now proposing a new parameter for preoperative evaluation, the DNA content of cells obtained from the cancer by percutaneous aspiration. Using absorption cytometry on resected tissues, they observed that the tumor cell characteristics of aneuploidy (60% of their patients) and high proliferative index were independent prognostic indices for shortened survival and unlikelihood of cure despite apparent surgical resectability. Furthermore these indices are said to be even more predictive than tumor size or the number of lymph nodes containing cancer. Allison et al. raise the question of whether such measurements "may eventually allow the identification of patients with pancreatic cancers which are 'resectable' by currently employed criteria, but who are not curable and for whom pancreatoduodenectomy provides little or no real benefit."[14] Before accepting that leap to determination of therapy, possibly giving up potential benefits to some patients *a priori,* we should examine several issues.

Other groups have examined the DNA content, ploidy, and proliferative index of pancreatic cancer cells and come to varying conclusions. Alanen et al[15] studied 62 cases, of which 39% were diploid: 12 of 24 diploid tumors were resectable, in contrast to only 3 of 38 nondiploid tumors. They inferred that resectable pancreatic cancers are a subgroup with less aggressive biologic behavior and cytologic characteristics. Although overall patient survival was much better in diploid tumors, the survival curves were not corrected for the treatment mode. Similarly Eskelinen et al.[16] found 48% of 46 pancreatic cancers were diploid. Thirty-six per cent of the diploid cancers and 79% of aneuploid cancers were associated with lymph node metastases. No aneuploid tumors proved to be resectable, and survivals of greater than 1 year occurred only after resection. Weger et al,[17] however, found only 11% of 70 patients had diploid tumors: seven of eight diploid cancers were resectable, but in their series 24 of 62 nondiploid cancers were resectable. Only resectable cancers were cured (2 of 8 and 3 of 62). In still greater contrast, Baisch et al[18] found that ploidy had no prognostic value for survival in 47 patients, despite the fact that 85% were diploid.

The data from the Hopkins study are based on 47 cases of pancreatic cancer removed by a Whipple-type pancreatoduodenectomy between 1975 and 1988. There were 89 patients so treated at that institution between 1969

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and 1990,[9] but we do not know how this subgroup was selected. We also do not know whether adjuvant radiation or chemotherapy might have conferred any individual differences in survival. The DNA studies were performed by absorption cytometry rather than the more common technique of flow cytometry,[15,16,18] but a good case is made by the authors for the comparability and the choice. A more important caveat is that the study was performed using tissue blocks from resected specimens but that the proposed application is projected to the use of cytologic material from preoperative needle aspirates. Most previous studies of DNA content and cell ploidy in pancreatic cancer also have used resected tissues.[15,16,18] Because of the variability of individual cells and the distribution curve of the aggregated findings, Allison et al. required at least 300 cells per tumor to make their judgments. One issue then is whether sufficient numbers of cells are routinely obtained by fine-needle aspiration to perform the analysis with reliability, and another is whether the cells that are aspirated are representative of the entire population. Although DNA analysis on needle-aspirated cytologic material has been reported to correlate with that on tissue specimens in other types of tumors,[19,20] the method has been applied to pancreatic cancer in only one report found.[17] In that study Weger et al.[17] required at least 100 cells per slide for valid analysis and were able to complete the test in 70 of 75 specimens with reproducibility.

Allison et al. found, as have others, that bigger pancreatic cancers have a worse prognosis, and they identified a subgroup of aneuploid tumors greater than 2.5 cm in diameter in which the prognosis was so dismal as to engender nihilism. Their observations are based on postoperative measurements of the real cancer by the pathologist, however. Notoriously the apparent size of a pancreatic cancer by preoperative radiologic assessment may be grossly overestimated because of the mass effect of pancreatitis frequently surrounding even a small cancer. From a practical standpoint, it may be hard to localize the smallest cancers and to retrieve sufficient numbers of cells by needle aspiration.

There is also the problem of specific diagnosis. Clearly if the cytologic aspirate does not retrieve malignant cells, the test cannot be used. But what about cancers other than ductal adenocarcinoma of the pancreas, including ampullary and bile duct cancer or even islet cell carcinoma? In almost 20% of cases, it is not possible to distinguish among the alternatives before resection.[21] Although Baisch et al.[18] found five resected ampullary cancers all to be diploid, longer-surviving, but ultimately fatal, almost nothing is yet known about the cytometric characteristics of nonpancreatic periampullary cancers.

A still bigger concern relates to the act of needle aspiration itself. It is well recognized that intraoperative biopsy of pancreatic cancer frequently causes dissemination of malignant cells,[22] and growth of cancer along the needle tract has been reported after percutaneous needle aspiration.[23] Peritoneal washings have been found to contain malignant cells much more often in patients after percutaneous needle aspiration (75%) than in patients who have not been needled (19%).[13] There is thus the distinct possibility that needle aspiration of pancreatic cancer, whether for diagnosis or prognosis, may burn any possible bridge to cure. Although our preliminary observations suggest that 10 Gy radiation before needling may reduce the seeding, we do not know the effect of irradiation on the proliferative index or other cytometric characteristics.

Putting aside all of the concerns previously noted, the remaining and biggest problem is how to use the information once obtained. Specifically should DNA content be used to decide on nonoperative palliation instead of attempting pancreatoduodenectomy? In this regard the Hopkins group found three populations of survival curves: small (<2.5 cm) diploid tumors were best; larger diploid and small aneuploid tumors intermediate; and larger aneuploid tumors (>2.5 cm) were by far the worst. It is for the latter subgroup that they entertain the hypothesis of abandoning resection. Can we really say there was no benefit conferred by resection in this worst-risk group, no cures, no prolongation of survival, no improvement of quality of life? There is evidence that resection does increase survival time,[2] and it is now believed that resection probably offers the best palliation,[5,7] especially in light of the lower surgical morbidity and mortality rates now to be expected. Within the present Hopkins study, there were patients with aneuploid cancers who survived 15, 16, 21, 27, and 42 months (the latter patient had a 2.5-cm tumor). Whether resection of the primary cancer also confers greater sensitivity to adjuvant therapy also remains to be examined.

It may be premature to accept defeat by pancreatic cancer even in the worse-case groups. There is some reason to believe that the nature of pancreatic cancer may be changing. Its incidence, which had risen steadily for decades, leveled off about 1973.[1] It is since then that reported cure rates after pancreatoduodenectomy (adjusted for surgical mortality and stage at presentation) have risen.[6–9] We do not know whether there have been changes in the patterns of ploidy or proliferative index during this period. We cannot see yet where the trend will end or whether new treatment initiatives such as preoperative and intraoperative irradiation[2] will have an effect. Perhaps for now absorption and flow cytometry data should be used prospectively only to stratify patients rather than as a gatekeeper for resection. If some populations of malignant cells (*i.e.*, aneuploid or proliferating) turn out to be more susceptible to cytotoxic therapy, studies of aspirated cells could become even more important in guiding treatment of unresectable cancers as well.

References

1. National Cancer Institute. Annual Cancer Statistics Review: 1973–1988. National Institutes of Health Pub #:90-2789 1990.
2. Warshaw AL, Swanson RS. Pancreatic cancer in 1988: possibilities and probabilities. Ann Surg 1988; 208:541-553.
3. Lea MS, Stahlgren LH. Is resection appropriate for adenocarcinoma of the pancreas? Am J Surg 1987; 154:651-654.
4. Crile G Jr. The advantages of bypass operations over radical pancreatoduodenectomy in the treatment of pancreatic carcinoma. Surg Gynecol Obstet 1970; 130:1049-1053.
5. Pellegrini CA, Heck CF, Raper S, Way LW. An analysis of the reduced morbidity and mortality rates after pancreaticoduodenectomy. Arch Surg 1989; 124:778-781.
6. Crist DW, Sitzmann JV, Cameron JL. Improved hospital morbidity, mortality, and survival after the Whipple procedure. Ann Surg 1987; 206:358-365.
7. Trede M, Schwall G, Saeger HD. Survival after pancreatoduodenectomy: 118 consecutive resections without an operative mortality. Ann Surg 1990; 211:447–458.
8. Grace PA, Pitt HA, Tompkins RK, et al. Decreased morbidity and mortality after pancreatoduodenectomy. Am J Surg 1986; 151: 141-148.
9. Cameron JL, Crist DW, Sitzmann JV, et al. Factors influencing survival after pancreatoduodenectomy for pancreatic cancer. Am J Surg 1991; 161:120-125.
10. Andersen JR, Sorensen SM, Kruse A, et al. Randomised trial of endoscopic endoprosthesis versus operative bypass in malignant obstructive jaundice. Gut 1989; 30:1132-1135.
11. Warshaw AL, Tepper JE, Shipley WU. Laparoscopy in the staging and planning of therapy for pancreatic cancer. Am J Surg 1986; 151:76-80.
12. Warshaw AL, Gu ZY, Wittenberg J, Waltman AC. Preoperative staging and assessment of resectability of pancreatic cancer. Arch Surg 1990; 125:230-233.
13. Warshaw AL. Implications of peritoneal cytology for staging of early pancreatic cancer. Am J Surg 1991; 6:26-30.
14. Allison DC, Bose KK, Hruban RH, et al. Pancreatic cancer cell DNA content correlates with long-term survival after pancreatoduodenectomy. Ann Surg 1991; 214:648-656.
15. Alanen KA, Joensuu H, Klemi PJ, Nevalainen TJ. Clinical significance of nuclear DNA content in pancreatic carcinoma. J Pathol 1990; 160:313-320.
16. Eskelinen M, Lipponen P, Collan Y, et al. Relationship between DNA ploidy and survival in patients with exocrine pancreatic cancer. Pancreas 1991; 6:90–95.
17. Weger AR, Glaser KS, Schwab G, et al. Quantitative nuclear DNA content in fine needle aspirates of pancreatic cancer. Gut 1991; 32:325-328.
18. Baisch H, Kloppel G, Reinke B. DNA ploidy and cell-cycle analysis in pancreatic and ampullary carcinoma: flow cytometric study of formalin-fixed paraffin-embedded tissue. Virchows Arch [A] 1990; 417:145-150.
19. Eneroth CM, Zetterberg A. The relationship between the nuclear DNA content smears of aspirates and the prognosis of mucoepidermoid carcinoma. Acta Otolaryngol 1975; 80:429–433.
20. Fallenius AG, Auer GU, Carstensen JM. Prognostic significance of DNA measurements in 409 consecutive breast cancer patients. Cancer 1988; 62:331-341.
21. Martin FM, Rossi RL, Dorrucci V, et al. Clinical and pathologic correlations in patients with periampullary tumors. Arch Surg 1990; 125:723-726.
22. Weiss SM, Skibber JM, Mohiuddin M, Rosato FE. Rapid intra-abdominal spread of pancreatic cancer: influence of multiple operative biopsy procedures. Arch Surg 1985; 120:415–416.
23. Ferrucci JT, Wittenberg J, Margolies MN, Carey RW. Malignant seeding of the tract after thin-needle aspiration biopsy. Radiology 1979; 130:345-346.

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WHAT'S NEW IN GENERAL SURGERY

Pancreatic Cancer Cell DNA Content Correlates With Long-term Survival After Pancreatoduodenectomy

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The DNA content of 47 adenocarcinomas arising in the head of the pancreas from patients who had undergone successful pancreatoduodenectomy was measured. The DNA measurements of each tumor were made without knowledge of the clinical course by absorption cytometry performed on Feulgen-stained nuclei that had been disaggregated from pancreatic cancer tissue blocks. Forty-seven evaluable DNA distributions were obtained from specimens taken between 1975 and 1988. Of the 47 tumors, 19 (40%) were diploid and 28 (60%) were aneuploid cancers. The 19 patients with diploid cancers had a median survival time of 25 months. Median survival of the 28 patients with aneuploid cancers was 10.5 months. This difference was statistically significant ($p = 0.003$). A multivariate life table regression analysis demonstrated that the ploidy and proliferative index as determined by absorption cytometry were independent prognostic factors, as strong as or stronger than the number of positive nodes and tumor size. Thus cellular DNA content appears to be one of the most important predictors of survival in patients with adenocarcinoma of the head of the pancreas who have successfully undergone a pancreaticoduodenectomy.

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THERE IS CONTROVERSY as to what constitutes optimal therapy for patients with pancreatic cancer. Advocates of aggressive management point out that the operative mortality rate for pancreatoduodenectomy is falling. In addition there seems to be a small (2.5% to 20%) but consistent group of 5-year survivors among patients undergoing successful pancreatoduodenectomy for this disease.[1–7] The value of surgically exploring all patients who are thought to potentially have resectable pancreatic cancers has been questioned.[8–11] It is estimated that less than 1% of all of the patients with pancreatic cancer will actually survive for 5 years.[5,8–12] Even when newer preoperative staging modalities are used to screen for resectable cancers, palliative operations are still being performed on the majority of patients. Many of these patients who undergo surgical exploration and palliative bypass might be better palliated with less morbidity, deaths, hospital time, and expense by the use of percutaneously or endoscopically placed biliary stents.[13,14] If one could determine at the time of patient presentation which patients had a pancreatic tumor with biology favoring long-term survival, perhaps just these patients could be explored, leaving the larger group to be palliated nonoperatively.

The DNA contents of most pancreatic cancers can be measured before operation by absorption cytometry on cancer cells obtained by fine-needle aspiration.[15–21] Thus tumor DNA content could provide useful preoperative prognostic information for patients with pancreatic cancer. To evaluate this possibility, a retrospective study of the DNA content of 47 pancreatic cancers was carried out in patients who had undergone a pancreatoduodenectomy. By using standard life table methods, the effect of the measured DNA content on survival was determined.

Materials and Methods

Selection of Cases for Study

All patients who underwent pancreatoduodenectomy at this institution between 1975 and 1988 and from whom

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adequate tissue specimens were available were evaluated for inclusion in this study. During this period pancreatoduodenectomy was performed on all patients who were found at surgical exploration to be free of distant metastatic disease and whose tumors did not invade the adjacent major vascular structures. The microscopic slides from all cases were reviewed by two of us (RHH and JKB). Four criteria were established for inclusion of a case in this study. First there had to be evidence of origin in the head of the pancreas. Second the neoplasm had to have a malignant histology. Third the neoplasm had to demonstrate both epithelial and glandular differentiation. Fourth only patients who survived surgery were included. The first criterion could be fulfilled in one of two ways: (1) the neoplasm contained an *in situ* component in the pancreatic ducts, or (2) the bulk of the neoplasm was present within the head of the pancreas. Cases in which an *in situ* component was identified solely within the bile duct or duodenum were excluded. Occasional cases in which there was apparent *in situ* carcinoma in both the pancreatic and bile duct were included only if the tumor was clearly centered in the pancreatic parenchyma rather than around the bile duct. To fulfill the second criterion, that of a malignant histology, the neoplasm had to have stromal, perineural, or vascular/lymphatic invasion. To fulfill the third criterion, the neoplasms had to show evidence of epithelial differentiation and lumen formation by light microscopy. In cases in which the carcinoma was poorly differentiated, the glandular differentiation was confirmed with the mucicarmine stain. Fifty-one pancreatic cancers were identified that fulfilled these criteria. The cells from four of these neoplasms retained insufficient Feulgen stain for adequate DNA measurements, leaving evaluable DNA distributions from 47 of the pancreatic cancers. Two cases were mixed adenocarcinomas–neuroendocrine tumors, and one case was a mixed adenocarcinoma-small cell carcinoma. The remaining 44 neoplasms were pure adenocarcinomas.

Cell Preparation and DNA Measurements

A modification of the basic method of cell preparation of Hedley and co-workers was employed.[22,23] The following consecutive sections were cut from the cancer-containing tissue blocks: a 5-μm section, at least three 50- to 100-μm sections, and then a 5-μ section. The two 5-μ sections were stained with hematoxylin and eosin for confirmation of the presence of pancreatic cancer cells in the 50- to 100-μm sections. The 50- to 100-μm sections were then rehydrated in a sequence of two 15-minute washes in xylene, followed by washes in ethanol/xylene (50:50) to 100%, 95%, 70%, and 50% ethanol in water at room temperature. The sections were then incubated overnight in 0.25% Trypsin solution (Grand Island Biological Company, Grand Island, NY) containing 1.5 mm spermine tetrahydrochloride (Sigma, St. Louis, MO) at 37 C with shaking. After vortexing and filtration over a 60-μm mesh nylon screen, the single cells and nuclei obtained were placed onto slides along with chicken erythrocytes as an internal standard for DNA content. The cells were fixed in 1% paraformaldehyde and then stained by the Feulgen reaction. The amount of Feulgen stain bound to each nucleus is proportional to the DNA content of that nucleus. Acid hydrolysis for Feulgen staining was performed in 4 N NaCl at 28 C for 1 hour, followed by Feulgen-staining in Schiff's reagent (Sigma, St. Louis, MO) for 1 hour at room temperature.[24] Absorption cytometric DNA measurements were made with a Vickers M85 microdensitometer (Vickers Instruments, Malden, MA) with a 100×, 1.25 NA achromatic lens. A 0.4-μm spot was used for the absorbance measurements. Absorbance measurements were made at light wavelengths between 570 and 615 nm, with a spectral bandwidth of 15 nm and a 4% instrument glare level,[24] and the integrated absorbances were automatically corrected for optical errors due to stain darkness.[25]

The absorption-cytometric DNA measurements on each slide were made without knowledge of the survival status of the patient from whom the tumor was taken. For generation of DNA distributions, DNA measurements on at least 300 cells per tumor were performed in randomly selected microscope fields. After disaggregation, it was possible to morphologically distinguish many nontransformed lymphocytes, polymorphonuclear leukocytes, and fibroblasts. We did not measure the DNA contents of all of these cells, but selectively performed absorption cytometric measurements on intact nuclei showing epithelioid, or indeterminant, morphologic features. The DNA contents of some adjacent chicken erythrocytes, as well as nontransformed host lymphocytes and polymorphonuclear leukocytes, were measured in each microscopic field to establish internal standards for DNA content.[24,25]

The DNA histograms obtained by absorption cytometry separated into two groups. One group was observed to contain a single major peak that contained morphologically identifiable stromal and inflammatory cells as well as tumor cells, which had a diploid DNA content (2C) and was assigned a DNA index of 1.0. Aneuploid tumors, which formed the second group, consisted of specimens in which distinct peaks in addition to the diploid peak could be identified. The DNA index of the aneuploid tumors was calculated as the ratio of the DNA content of the abnormal DNA stemline to that of the diploid peak. Two of the cancers were classified as tetraploid (DNA index = 2 and having >10% S- and G2/M-phase tetraploid cells). These tetraploid cancers were grouped with the aneuploid tumors. The DNA measurements also provide a gauge of the proportion of tumor cells that are in the different phases of the cell cycle (G0/G1, S, and G2/M phases), and these proportions were calculated from rectangular DNA distributions.[26] The proliferative index (PI) is the proportion of tumor cells in the S and G2/M phases of the cell cycle. Finally absorption cytometric DNA measurements can lead to the detection of rare tumor cells with DNA contents far above that of the G0/G1-, S-, and G2/M-phase cells of the "main" tumor stemline.[27] Because the presence of such cells might portend for a poor patient prognosis, the percentage of such ">G2/M cells" in each tumor also was measured.

Statistical Methods

The major statistical endpoint of this study was the duration of survival. Nonparametric estimates of the probability of survival were made by the product-limit method.[28] Differences between survival distributions were assessed for statistical significance by means of the log-rank statistic.[29] The prognostic effect of DNA measurements and of other dichotomous variables was expressed as a *hazard ratio*, in other words, the risk of death in those with the variable divided by the risk of death in those without. Thus hazard ratios greater than 1.0 imply an increased risk for those with the prognostic factor, and ratios less than 1.0 imply a decreased risk. For continuously distributed prognostic variables (*e.g.*, age), the hazard ratio was expressed per unit change (*e.g.*, change per year of increased age). Hazard ratios were estimated using the proportional hazards regression model.[30] To estimate hazard ratios while simultaneously controlling for other prognostic factors, we used the multivariate proportional hazards regression model.[30] The multivariate models focused on both the overall strongest independent predictors and the strongest independent preoperative predictors. To demonstrate multivariate analyses clinically, patients were classified into risk groups based on statistically significant prognostic factors. These risk groups have significantly different prognoses as a consequence of their arising from the multivariate analyses. All p values reported are two-sided.

Results

Patients

Of the 47 patients for whom we obtained interpretable histograms, 22 were men and 25 were women. The ages of these patients ranged from 33 to 74 years at the time of tumor resection. There were 37 white and 10 black patients. Current follow-up data were available for all patients (Table 1). All patients were discharged from the hospital after surgery except for one who died with liver metastases 2 months after resection. The liver metastases in this case were confirmed by postmortem examination. Thirty-four of the thirty-five patient deaths were directly attributable to recurrent cancer, with one patient dying of an apparent cerebrovascular accident 4 months after resection.

TABLE 1. *Age, Sex, Race, and Postresectional Survival Times (mo) of Pancreatic Cancer Patients Whose Tumor DNA Contents Were Measured*

Age Range at Resection	Race		Dead	Alive
	Black	White		
M				
50–59	2		3, 12	
60–69	2		2, 9	
30–39		2	10	24
40–49		1	9	
50–59		6	8, 11, 12, 13	15, 23
60–69		7	5, 8, 8, 32	21, 27, 127
70–79		2	12, 19	
F				
40–49	1		44	
50–59				
60–69	4		4, 6, 9, 23	
70–79	1			55
40–49		2	5, 11	
50–59		6	10, 12, 24, 26, 76	49
60–69		8	5, 12, 13, 23, 26, 69	16, 29
70–79		3	5	42, 44

Pancreatic Cancer DNA Contents, Pathology, and Patient Survival

The quality of the DNA histograms generated from the archival material was generally good, although the older material stained less intensely than did the more recent samples. This was demonstrated by an decreased absorbance ratio of the G0/G1 human diploid cells to chicken erythrocytes for nuclei harvested from the older tissue blocks. It also was indicated by the fact that evaluable DNA distributions were obtained from only three of seven tissue blocks from specimens obtained before 1980, whereas all of the 44 tissue blocks obtained in 1980 or later yielded evaluable distributions. There were 19 diploid and 28 aneuploid pancreatic cancers (Tables 2 and 3). None of the aneuploid cancers had DNA indices of less than 1. Twenty-six of twenty-eight aneuploid cancers had G0/G1 DNA peaks with cell numbers that made up 9% or more of their respective G0/G1 diploid DNA peaks (range, 9% to 91%, average of 39%). Two tumors were considered to be aneuploid on the basis of morphologically similar cells clustered at G0/G1 DNA peaks with indices of 1.69 and 1.74, even though these relatively rare aneuploid G0/G1 cells had cell numbers that were only 4%

TABLE 2. *Tumor Size, Cell Cycle Parameters, and Survival Status of Patients With Diploid Cancers After Successful Pancreatoduodenectomy*

DNA Index	Survival Time (mo)	Survival Status	Size (cm)	Nodes	% G1	% S	% G2/M	% > G2/M
1.00	5	Dead	3	2/7	84	7	9	0.3
1.00	8	Dead	3	1/25	86	8	4	2
1.00	11	Dead	1.5	11/25	88	7	5	0.3
1.00	12	Dead	3.5	1/7	95	2	3	0
1.00	12	Dead	3.5	3/12	92	4	4	0
1.00	19	Dead	2	2/5	97	0	3	0
1.00	23	Alive	2	0/10	87	10	2	1
1.00	23	Dead	4	4/8	90	4	6	0
1.00	24	Alive	4	2/10	94	1	5	0
1.00	25	Dead	5	2/14	80	4	15	1
1.00	26	Dead	7	0/5	97	2	1	0.3
1.00	29	Alive	2.5	0/6	88	2	10	0.3
1.00	44	Alive	2	0/0	91	1	9	0
1.00	44	Dead	1.5	0/27	90	5	5	0
1.00	49	Alive	3	1/12	82	9	7	1
1.00	55	Alive	2	0/8	94	2	4	0
1.00	69	Dead	1.5	2/14	95	3	2	0
1.00	76	Dead	2	0/21	94	5	1	0
1.00	127	Alive	4	0/16	90	4	5	1

and 6%, respectively, of their diploid G0/G1 DNA peaks. Strong support for the aneuploid classification of these tumor was provided by the finding in both cases of morphologically identical cells with tightly clustered DNA contents at the expected G2 DNA values for these tumors, as well as the presence of cells with DNA values within the appropriate ranges for their S-phase compartments.

The PI of the diploid cancers ranged from 3% to 16% (Table 2), whereas the PIs of the aneuploid cancers ranged from 5% to 38% (Table 3). Nine of the nineteen diploid

TABLE 3. *Tumor Size, Cell Cycle Parameters, and Survival of Patients With Aneuploid Tumors After Successful Pancreatoduodenectomy*

DNA Index	Survival Time (mo)	Survival Status	Size (cm)	Nodes	% GI	% S	% G2/M	% > G2/M
1.57	2	Dead	3	4/6	72	14	10	4
1.82	3	Dead	4.5	2/13	92	7	1	0
1.76	4	Dead	4	1/7	83	16	0	1
1.89	5	Dead	4	0/5	72	8	15	5
1.81	5	Dead	3	7/11	70	17	13	0
2.25	5	Dead	2.5	3/10	85	7	7	1
1.81	6	Dead	4.5	6/13	65	20	14	2
1.73	8	Dead	6.5	2/19	61	32	4	3
1.45	8	Dead	5.5	6/60	78	21	1	0
1.56	9	Dead	3	0/0	60	19	19	1
2.06*	9	Dead	4	2/5	86	9	2	3
1.75	9	Dead	2	5/17	63	13	25	0
1.69	10	Dead	5	1/6	72	16	12	0
1.88	10	Dead	3.5	3/6	89	8	3	0
1.87	11	Dead	6	2/4	78	14	7	2
1.92	12	Dead	3	0/8	80	10	10	0
1.74	12	Dead	2.5	1/8	83	13	2	2
1.56	12	Dead	2.5	2/14	69	19	8	4
1.67	13	Dead	1.5	0/3	76	9	12	2
1.90	13	Dead	3	1/12	86	12	1	1
1.61	15	Alive	0.5	0/0	89	0	11	0.5
1.98*	16	Alive	2	0/7	83	3	13	0
1.67	21	Alive	2	0/10	81	12	6	1
1.79	23	Dead	2	2/3	91	5	4	0.7
1.85	24	Dead	3.5	9/19	82	11	7	0
1.84	27	Alive	1.5	2/11	90	9	1	0
1.66	32	Dead	1	0/13	77	16	6	1
1.79	42	Alive	2.5	3/8	95	3	2	0

* Tetraploid cancers.

tumors had a small percentage of cells (0.3% to 2%, Table 2) with DNA contents higher than the main stemline ($>G_2M$), whereas 17 of the 28 aneuploid cancers had such $>G_2/M$ cells (0.5% to 5%, Table 3).

Figure 1A and B shows photomicrographs of two different pancreatic cancers. Both tumors show invasive adenocarcinomas with marked nuclear hyperchromasia. Although both appear relatively similar histologically, Figure 2A and B illustrates that the DNA distributions of these pancreatic cancers are in fact quite different. The tumor in Figures 1A and 2A had a diploid DNA content with a G0/G1 peak having a DNA index of 1, and a corresponding G2/M peak with a DNA index of 2. This tumor had a relatively high PI of 16% (Fig. 2A). The tumor in Figures 1B and 2B was aneuploid, with a DNA index of 1.56 and a PI of 27%. The high PI of the diploid tumor may be the explanation for the hyperchromatic staining of many of the cell nuclei in this tumor, which resembled the intense nuclear staining of the aneuploid cancer. The DNA measurements allowed separation of two tumors that could not be separated histologically into different classes. It is of interest that the patient with the aneuploid cancer (Figs. 1B, 2B) succumbed to progressive disease 12 months after resection, whereas the patient with the diploid cancer (Figs. 1A, 2A) has remained tumor free for more than 4 years after resection.

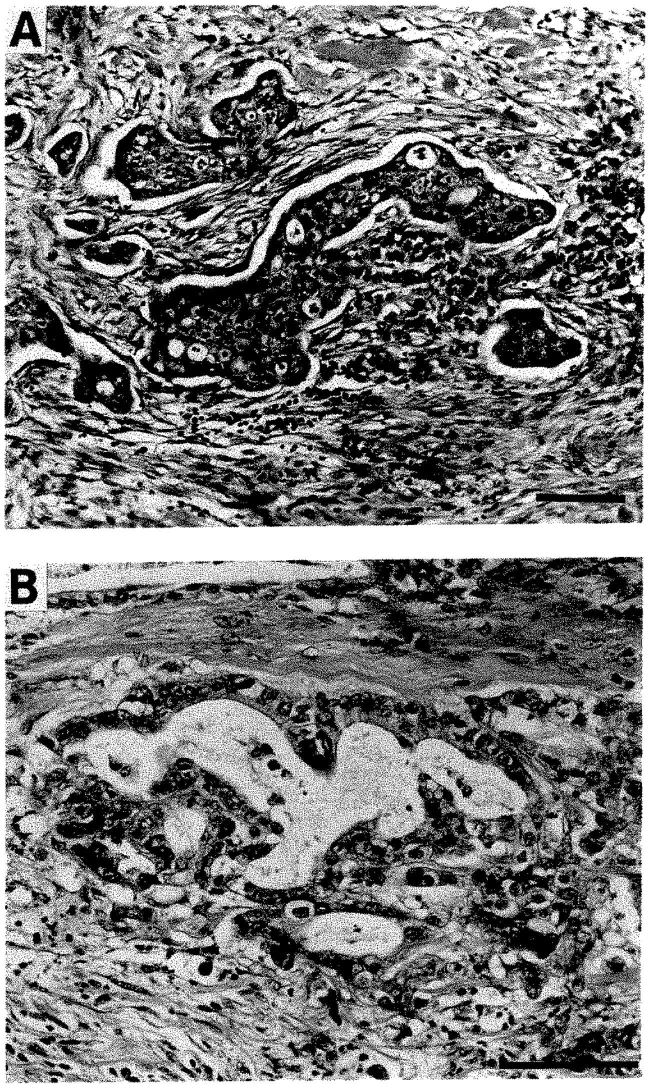

FIG. 1A and B. Hematoxylin and eosin–stained 5-μm sections of pancreatic adenocarcinomas in two pancreatoduodenectomy specimens. A, Section of a 3-cm tumor resected from a 59-year-old white woman shows deep invasion, marked nuclear atypia, and hyperchromasia. B, Section of a 2.5-cm tumor resected from a 50-year-old white man also shows deep invasion, marked nuclear atypia, and hyperchromasia. *Bar,* 100 μm.

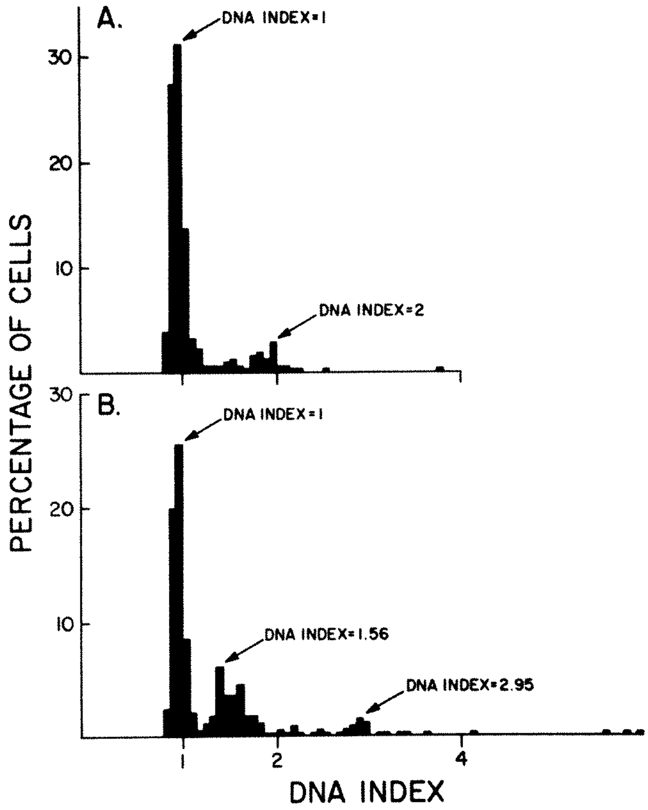

FIG. 2A and B. DNA distributions of the two pancreatic tumors shown in Fig. 1. A, DNA distribution of the tumor shown in Fig. 1A. This tumor was diploid (DNA index, 1), with a PI of 16%. B, DNA distribution of the tumor shown in Fig. 1B. This tumor contains both diploid cells (DNA index, 1) and an aneuploid tumor stemline (DNA index, 1.56), with a PI of 27%. This patient died of progressive pancreatic cancer 12 months after resection, whereas the patient with the diploid cancer remains tumor-free 4 years after resection.

Univariate analysis demonstrated that the hazard of death for patients with aneuploid tumors was 3.3 times greater than that for patients with diploid tumors (Table 4). This hazard ratio is significantly greater than 1 ($p = 0.003$). For the patients with diploid pancreatic cancers, the median survival time was 25 months, whereas patients with aneuploid tumors had a median survival of only 10.5 months. The percentage of S-phase cell, the percentage of cells with >G_2M DNA content, a high PI, and the presence of lymph node metastases all had unfavorable prognostic influences in univariate analysis when patients with both diploid and aneuploid tumors were considered together or separately (Table 4). As expected, the univariate analysis also showed that age, race, sex, and year of resection were *not* prognostic for long-term survival (Table 4).

Several multivariate models were used for prediction of survival time (Table 5). Aneuploid *versus* diploid DNA content remained highly prognostic even after adjustment for tumor size, PI, and nodal involvement (Table 5). The risk of death for patients with aneuploid tumors appeared to be approximately four times that for patients with diploid tumors, even after adjustment for the size of the tumor. After ploidy status and tumor size were accounted for, the presence of cells with DNA contents >G2/M, age, sex, and race did not appear to be statistically significant prognostic variables. Patients diagnosed and treated more recently did not have a significantly more favorable prognosis than did earlier patients (Table 5). The strongest preoperative prognostic factors were ploidy status, tumor size, and PI.

The estimated 3-year survival rates for patients with diploid and aneuploid cancers were 51% and 8%, respectively. The difference in survival is shown graphically in Figure 3. This difference is statistically significant ($p < 0.003$, Table 4). Placement of the two patients with the tetraploid cancers in the diploid rather than the aneuploid group did not appreciably alter the results of the statistical analysis. Also removal of the one patient who apparently did not die of recurrent pancreatic cancer from consideration did not substantially change the results of the statistical analysis.

TABLE 4. *Estimated Hazard Ratios for Death, With Significance Levels, When Prognostic Factors are Considered Individually*

Variable	Hazard Ratio	95% Confidence Limits	p*
Aneuploid *vs.* diploid	3.28	1.48–7.24	0.003
% G0/G1	0.93	0.89–0.96	<0.001
% S	1.11	1.05–1.16	<0.001
% G2/M	1.05	0.98–1.13	0.14
% > G2/M	1.46	1.12–1.89	0.004
Σ (S + G2/M + >G2/M)†	1.08	1.04–1.12	<0.001
PI (S + G2/M)†	1.08	1.04–1.12	<0.001
Tumor size/cm‡	1.28	1.04–1.56	0.02
>2.5 cm *vs.* ≤ 2.5 cm	2.75	1.36–5.60	0.005
Positive nodes/node§	1.15	1.02–1.28	0.02
Nodes, positive vs. negative	3.26	1.42–7.62	0.006
Age/year	0.99	0.95–1.02	0.49
White *vs.* nonwhite	0.50	0.23–1.08	0.08
F *vs.* M	0.86	0.44–1.71	0.67
Year of diagnosis	1.02	0.92–1.13	0.69

* p value for hypothesis that the hazard ratio equals 1.0.
† Hazard ratio for each 1% of PI or Σ(S + G2/M + >G2/M).
‡ Hazard ratio for each centimeter of tumor diameter.
§ Hazard ratio for each positive lymph node.
PI, proliferative index.

TABLE 5. *Estimated Hazard Ratios for Death, With Significance Levels, for Multivariate Proportional Hazards Regression Models**

Term	Hazard Ratio	95% Confidence Limits	p†
Aneuploid	2.85	1.10–7.34	0.03
PI	1.05	1.00–1.10	0.07
Tumor > 2.5 cm	2.98	1.40–6.33	0.01
Aneuploid	4.88	1.96–12.16	<0.001
Year of diagnosis	0.95	0.84–1.08	0.44
Tumor size/cm	1.38	1.11–1.70	0.004
Nodes, positive *vs.* negative	2.44	1.01–5.87	0.05
Aneuploid	2.48	1.00–6.27	0.05
Tumor > 2.5 cm	2.47	1.14–5.35	0.02
PI	1.05	1.00–1.10	0.03
Aneuploid	4.18	1.83–9.54	<0.001
Tumor > 2.5 cm	3.47	1.66–7.24	<0.001

* Each panel represents a separate multivariate model.
† p value for hypothesis that the hazard ratio equals 1.0.
PI, proliferative index.

Relationships Between the DNA Measurements, Tumor Size, and Patient Survival

Table 5 shows that tumor ploidy levels and size were statistically significant and independent prognostic variables for the length of survival after successful resection for pancreatic cancer. Based on the multivariate analysis in Table 5 (last panel), risk groups can be identified using ploidy and tumor size. Figure 4 is a Kaplan-Meier plot of the survival times of the risk groups with small and large diploid and aneuploid cancers. It can be seen that the patients with small (≤2.5 cm) diploid cancers have 2-

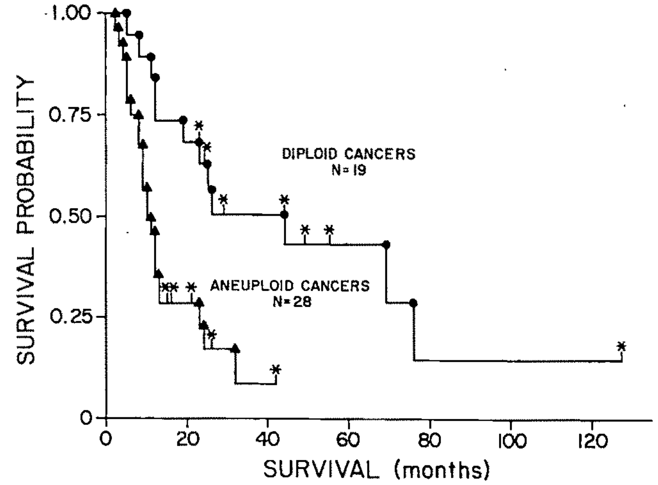

FIG. 3. Kaplan-Meier survival curves for the 19 patients with diploid (●) and the 28 patients with aneuploid pancreatic carcinoma (▲) who survived pancreatoduodenectomy. Time of last follow-up of surviving patients (*).

year survivals of 78%. The patients with small (≤2.5 cm) aneuploid and large (>2.5 cm) diploid cancers had intermediate 2-year survivals of 44% and 50%, respectively. Statistical tests of these differences are shown in Table 5, last panel. Only 1 of the 16 patients with aneuploid pancreatic cancers greater than 2.5 cm survived longer than 1 year, and this patient succumbed to recurrent disease 24 months after resection (Fig. 4). None of the nine patients with aneuploid tumors 4 cm or larger survived for longer than 11 months after resection (Table 3). Because the survival times plotted do not include the time of hospitalization for surgery (average of 1 month) and subsequent admissions for the complications of progressive disease and terminal care, it would seem that the patients with large aneuploid pancreatic cancers have very limited lifespans outside of the hospital after resection.

Discussion

We undertook the present work in an effort to determine whether cellular DNA content, as measured by absorption cytometry, is a valid prognostic indicator for carcinoma of the pancreas. This technique of DNA measurement, rather than flow cytometry, was selected because it can readily be applied to pancreatic cancer cells obtained preoperatively by fine needle aspiration.[21] Also absorption-cytometric DNA measurements seem to pose fewer problems with the artifactual interpretation of cellular debris as S-phase cells and the loss of rare cell populations from the DNA distribution.[27,31]

Measurement of DNA content, performed either by absorption or flow cytometry, allows determination of whether a tumor is diploid or aneuploid and also indicates the proportion of proliferating tumor cells (expressed as PI). On theoretical grounds, one would suppose that patients with aneuploid tumors, or tumors with a high PI, should have a worse prognosis; however, clinical experience with measurements of DNA content for a wide variety of human cancers has shown that this is not always the case. Many clinically benign tumors have an aneuploid DNA content.[32–36] For carcinomas of the thyroid and gallbladder, carcinoid tumors, and node-positive breast cancers, ploidy levels or the PI give little independent prognostic information.[37–40] For colorectal cancers,[41–44] melanoma,[45] and node-negative breast cancers,[46] however, the DNA measurements provide important and useful prognostic information. Surprisingly for some cancers, such as neuroblastomas, aneuploidy in a tumor is a favorable prognostic sign.[47] Thus the value of DNA content as a prognostic indicator must be assayed independently for each type of human cancer.

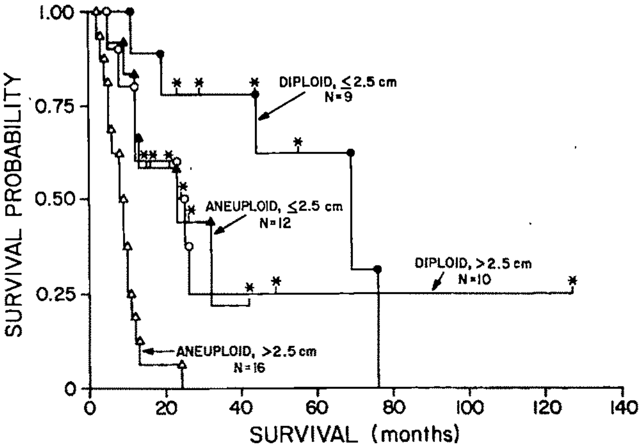

FIG. 4. Kaplan-Meier survival curves for the 9 patients with small (≤ 2.5-cm) diploid pancreatic tumors (●); the 12 patients with small aneuploid carcinomas (▲); the 10 patients with large (> 2.5-cm) diploid cancers (○); and the 16 patients with large aneuploid cancers (△). Time of last follow-up of surviving patients (*).

In the present study, tumor aneuploidy correlated with poor survival (Tables 4, 5). Only 8% of the patients with aneuploid cancers survived for 3 years, whereas 51% of the patients with diploid cancers were alive at 3 years (Fig. 3). Multivariate analyses demonstrated that ploidy levels (aneuploid *vs.* diploid DNA status), the PI, nodal involvement, and the size of the carcinoma were statistically significant prognostic indicators (Table 5). The age, sex, and race of the patients were not independent prognostic indicators of survival after correction for tumor ploidy level, the PI, nodal involvement, and tumor size.

Joensuu et al.[48] found in a flow cytometric study that only 3 of 15 resected pancreatic cancers had aneuploid DNA content, whereas 35 of 47 nonresected pancreatic cancers were aneuploid. Although none of the patients in this series survived longer than 2 years, the patients with diploid pancreatic cancers lived longer than patients with aneuploid cancers. This finding led these authors to conclude that the modest survival advantage observed for the group of patients undergoing surgical resection was due not to an actual benefit of surgery, but to the fact that the tumors with more intrinsically favorable biologic behavior were being selected for resection.[48]

In a recent flow cytometric and image analysis DNA (Feulgen) study, Weger et al.[49] found that 76 of 77 pancreatic cancers (50 of which were resected) had "nondiploid" DNA contents. Furthermore these authors concluded that patients with "triploid" DNA contents had an especially poor prognosis. We believe the high rate of aneuploid pancreatic cancers found by Weger et al., when compared with the rates of aneuploidy found in our study (Tables 3, 4) and that of Joensuu et al.,[48] may be due to technical artefacts. Selection of the relatively rare cells in diploid tumors with >G2/M DNA contents (Table 2) for image analysis DNA measurements could give an erroneous impression of aneuploidy. Also Weger et al.[49] analyzed material from tissue blocks obtained between 1972 and 1988. We found that Feulgen DNA measurements obtained from tissue blocks 8 years or older were often unreliable and showed widening of the 2C and 4C DNA peaks. Optical errors such as glare can cause dispersion of DNA measurements.[24,25] Finally flow cytometric studies of the DNA contents of disaggregated pancreatic cells can lead to spurious, "false aneuploid" peaks around the 2C and 4C DNA values secondary to autolysis of nuclei by pancreatic enzymes.[50] Thus it is possible that only the "triploid" cancers of Weger et al. are truly aneuploid. If this is the case, the relatively poor prognosis observed for this subset of patients[49] would be consistent with the poor survival results for patients with aneuploid pancreatic cancers found in our study (Fig. 3) and by Joensuu et al.[48]

The finding that patients with diploid cancers survived longer than patients with aneuploid cancers supports the hypothesis that the biologic behavior of pancreatic cancer plays a large part in the duration of patient survival.[48,49] We believe that this finding does not imply, as Joensuu et al. suggest, that resectional surgical therapy should not play an important role in treatment for the majority of pancreatic cancers for which the operation is possible.[48] Admittedly the two long-term deaths from recurrent pancreatic cancer, at 69 and 76 months after resection, leave open the possibility that at least some of the apparent benefits of surgery with regard to survival are due to lead time bias. Nevertheless there are five other patients in this series who are currently approaching or are alive more than 4 years after resection. We hope that at least some of these patients will be "cured," and there is one 10-year tumor-free survivor. Regardless of the eventual outcomes of these patients, however, one can only speculate what their clinical courses would have been if their cancers had not been resected.

A subset of patients with "large" (>2.5 cm) aneuploid cancers were identified who had uniformly poor survivals even after "curative" resections (Table 3, Fig. 4). Because DNA measurements by absorption cytometry can be performed on pancreatic cancer cells obtained by fine-needle aspiration,[24] the combination of these DNA measurements with preoperative imaging estimates of tumor size may have future potential for the selection of therapy for certain pancreatic cancer patients. Specifically the DNA and tumor size measurements may eventually allow the identification of patients with pancreatic cancers that are "resectable" by currently employed criteria, but who are not curable and for whom pancreatoduodenectomy provides little or no real benefit (Table 3, Fig. 4). Also these measurements may allow the identification of other pancreatic cancer patients with tumors currently judged "unresectable," for whom more extensive attempts at regional surgical resection, including resecting portions of the portal and superior mesenteric veins, could be justified. Finally this information may allow more accurate stratification of pancreatic cancer patients in controlled studies to determine which of these patients may benefit from adjuvant therapy.[51,52]

The clinical application of preoperative imaging techniques and DNA measurements as a guide for selecting patients for surgical resection at this time would be premature. The results of this retrospective study in which we work with fixed material should be confirmed and extended prospectively with a larger number of patients before the DNA measurements can be used with confidence as a basis for clinical decisions.

Acknowledgment

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References

1. Trede M. The surgical treatment of pancreatic carcinoma. Surgery 1985; 97:28–35.
2. Grace PA, Pitt HA, Tompkins RK, et al. Decreased morbidity and mortality after pancreatoduodenectomy. Am J Surg 1986; 151: 141–149.
3. Braasch JW, Deziel DJ, Rossi RL, et al. Pyloric and gastric preserving pancreatic resection: experience with 87 patients. Ann Surg 1986; 204:411–418.
4. Crist DW, Sitzmann JV, Cameron JL. Improved hospital morbidity, mortality, and survival after the Whipple procedure. Ann Surg 1987; 206:358–365.
5. Warshaw AL, Swanson RS. Pancreatic cancer in 1988: possibilities and probabilities. Ann Surg 1988; 208:541–553.
6. Michelassi F, Erroi F, Dawson PJ, et al. Experience with 647 consecutive tumors of the duodenum, ampulla, head of pancreas, and distal common bile duct. Ann Surg 1989; 210:544–556.
7. Pellegrini CA, Heck CF, Raper S, Way LW. An analysis of the reduced morbidity and mortality rates after pancreaticoduodenectomy. Arch Surg 1989; 124:778–781.
8. Crile G Jr. The advantages of bypass operations over radical pancreatoduodenectomy in the treatment of pancreatic carcinoma. Surg Gynecol Obstet 1970; 130:1049–1053.
9. Shapiro TM. Adenocarcinoma of the pancreas: a statistical analysis of biliary bypass versus Whipple resection in good risk patients. Ann Surg 1975; 182:715–721.
10. Gudjonsson B. Cancer of the pancreas: 50 years of surgery. Cancer 1987; 60:2284–2303.
11. Lea MS, Stahlgren LH. Is resection appropriate for adenocarcinoma of the pancreas? Am J Surg 1987; 154:651–654.
12. Connolly MM, Dawson PJ, Michelassi F, et al. Survival in 1001 patients with carcinoma of the pancreas. Ann Surg 1987; 206: 366–373.
13. Bornman PC, Harries-Jones EP, Tobias R, et al. Prospective controlled trial of transhepatic biliary endoprosthesis versus bypass surgery for incurable carcinoma of head of pancreas. Lancet I; 1986:69–71.
14. Shepherd HA, Royle G, Ross APR, et al. Endoscopic biliary endoprosthesis in the palliation of malignant obstruction of the distal common bile duct: a randomized trial. Br J Surg 1988; 75: 1166–1168.
15. Ferrucci JT Jr, Wittenberg J, Mueller PR, et al. Diagnosis of abdominal malignancy by radiologic fine needle aspiration biopsy. Am J Roentgenol 1980; 134:323–330.
16. Tatsuta M, Yamamoto R, Yamamura H, et al. Cytologic examination and CEA measurement in aspirated pancreatic material collected by percutaneous fine-needle aspiration biopsy under

ultrasonic guidance for the diagnosis of pancreatic carcinoma. Cancer 1983; 52:693–698.

17. Silverstein MD, Richter JM, Podolsky DK, Warshaw AL. Suspected pancreatic cancer presenting as pain or weight loss: analysis of diagnostic strategies. World J Surg 1984; 8:839–845.
18. Dickey JE, Haaga JR, Stellato TA, et al. Evaluation of computed tomography guided percutaneous biopsy of the pancreas. Surg Gynecol Obstet 1986; 163:497–503.
19. Taavitsainen M, Koivuniemi A, Bondestam S, et al. Ultrasonically guided fine-needle aspiration biopsy in focal pancreatic lesions. Acta Radiol 1987; 28:541–543.
20. Ekberg O, Bergenfeldt M, Aspelin P, et al. Reliability of ultrasound-guided fine-needle biopsy of pancreatic masses. Acta Radiol 1988; 29:535–539.
21. Schobl R, Dimmer V. Computergestutzte Diagnose an Feinnadelaspirationsbiopsien des Pankreas mittels automatisierter Mikroskopbildanalyse. Arch Geschwulstforsch 1989; 59:277–287.
22. Hedley DW, Friedlander ML, Taylor IW, et al. Method for analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry. J Histochem Cytochem 1983; 31: 1333–1335.
23. Schutte B, Reynders MMJ, Bosmon FT, Blijham GH. Flow cytometric determination of DNA ploidy level in nuclei isolated from paraffin-embedded tissue. Cytometry 1985; 6:26–30.
24. Allison DC, Ridolpho PF, Rasch EM, et al. Increased accuracy of absorption cytophotometric DNA values by control of stain intensity. J Histochem Cytochem 1981; 29:1219–1228.
25. Allison DC, Lawrence GN, Ridolpho PF, et al. Increased accuracy and speed of absorption cytometric DNA measurements by automatic corrections for nuclear darkness. Cytometry 1984; 5:217–227.
26. Allison DC, Chakerian M, Ridolpho P, et al. Combined flow and absorption DNA measurements of (3H) thymidine labelled tumour cells: I. Studies of MCa-11 cells grown as tumors *in vivo* and exponential cultures *in vitro*. Cell Tissue Kinet 1987; 20: 273–290.
27. Bose KK, Curley S, Smith WJ, Allison DC. Differences in the flow and absorption cytometric DNA distributions of mouse hepatocytes and tumor cells. Cytometry 1989; 10:388–393.
28. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. JASA 1958; 53:457–481.
29. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. JNCI 1959; 22:719–748.
30. Cox DR. Regression models and life tables. J R Stat Soc [B] 1972; 34:187–220.
31. Givan AL, Shenton BK, Carr TW. A correction required for calculation of DNA ratios in flow cytometric analysis of ploidy. Cytometry 1988; 9:271–274.
32. Anniko M, Tribukait B, Wersall J. DNA ploidy and cell phase in human pituitary tumors. Cancer 1984; 53:1708–1713.
33. van den Ingh HF, Griffioen G, Cornelisse CJ. Flow cytometric detection of aneuploidy in colorectal adenomas. Cancer Res 1985; 45:3392–3397.
34. Joensuu H, Klemi P, Eerola E. DNA aneuploidy in follicular adenomas of the thyroid gland. Am J Pathol 1986; 124:373–376.
35. Joensuu H, Klemi P. DNA aneuploidy in adenomas of endocrine organs. Am J Pathol 1988; 132:145–151.
36. Joensuu H, Klemi PJ, Alanen KA. Co-existence of two aneuploid stemlines in benign adenomas. Virchows Arch [A] 1989; 415: 175–180.
37. Tsushima K, Nagorney DM, Weiland LH, Lieber MM. The relationship of flow cytometric DNA analysis and clinicopathology in small intestinal carcinoids. Surgery 1989; 105:366–373.
38. Hamming JF, Schelfhout LJDM, Cornelisse CJ, et al. Prognostic value of nuclear DNA content in papillary and follicular thyroid cancer. World J Surg 1988; 12:503–508.
39. Donohue JH, Nagorney DM, Grant CS, et al. Carcinoma of the gallbladder: does radical resection improve outcome? Arch Surg 1990; 125:237–241.
40. Hedley DW, Rugg CA, Gelber RD. Association of DNA index and S-phase fraction with prognosis of node positive early breast cancer. Cancer Res 1987; 47:4729–4735.
41. Armitage NC, Robins RA, Evans DF, et al. The influence of tumour cell DNA abnormalities on survival in colorectal cancer. Br J Surg 1985; 72:828–830.
42. Scott NA, Wieand HS, Moertel CG, et al. Colorectal cancer: Duke's stage, tumor site, preoperative plasma CEA level, and patient prognosis related to tumor DNA ploidy pattern. Arch Surg 1987; 122:1375–1379.
43. Jones DJ, Moore M, Schofield PF. Prognostic significance of DNA ploidy in colorectal cancer: a prospective flow cytometric study. Br J Surg 1988; 75:28–33.
44. Heimann TM, Miller F, Martinelli G, et al. Significance of DNA content abnormalities in small rectal cancers. Am J Surg 1990; 159:199–203.
45. Kheir SM, Bines SD, Vonroenn JH, et al. Prognostic significance of DNA aneuploidy in stage I cutaneous melanoma. Ann Surg 1988; 207:455–461.
46. Clark GM, Dressler LG, Owens MA, et al. Prediction of relapse or survival in patients with node-negative breast cancer by DNA flow cytometry. N Engl J Med 1989; 320:627–633.
47. Taylor SR, Blatt J, Constantino JP, et al. Flow cytometric DNA analysis of neuroblastoma and ganglioneuroma. Cancer 1988; 62:749–754.
48. Joensuu H, Alanen KA, Klemi P. Doubts on "curative" resection of pancreatic cancer. Lancet 1989; I:953–954.
49. Weger AR, Falkmer VG, Schwab G, et al. Nuclear DNA distribution pattern of the parenchymal cells in adenocarinomas of the pancreas and in chronic pancreatitis. Gastroenterology 1990; 99:237–242.
50. Joensuu H, Alanen KA, Klemi PJ, Aine R. Evidence for false aneuploid peaks in flow cytometric analysis of paraffin-embedded tissue. Cytometry 1990; 11:431–437.
51. Kalser MH, Ellenberg SS. Pancreatic cancer: adjuvant combined radiation and chemotherapy following curative resection. Arch Surg 1985; 120:899–903.
52. Gastrointestinal tumor study group. Further evidence of effective combined adjuvant radiation and chemotherapy following curative resection of pancreatic cancer. Cancer 1987; 59:2006–2010.

Distinguishing Pancreatic Carcinoma From Other Periampullary Carcinomas by Analysis of Mutations in the Kirsten-*ras* Oncogene

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The prevalence of Kirsten (Ki)-*ras* gene mutations was studied in 105 paraffin-embedded tissues obtained from 40 patients with pancreatic cancer, 48 with bile duct carcinoma (19 distal, 6 middle, and 23 proximal), 16 with ampullary carcinoma and 1 with duodenal cancer, by *in vitro* amplification of target sequences by the polymerase chain reaction (PCR). With regard to pancreatic cancers, the authors' data confirm the very high frequency (88.6%) of Ki-*ras* gene mutations occurring at codon 12. Five pancreatic carcinomas did not contain the Ki-*ras* mutation and included rare types of histopathology. By histologic review after the examination of Ki-*ras* mutations through PCR, the diagnosis of four patients could be legitimately revised from other periampullary carcinoma to pancreatic carcinoma. In the ampullary carcinoma, the prevalence of mutations in Ki-*ras* codon 12 was 13.3%. Although there was a large difference in incidence of mutations between distal and middle or proximal bile duct carcinoma, the prevalence of mutations in bile duct carcinoma was limited to 19.6%. Unlike other approaches to diagnose periampullary carcinoma, detection of a mutation in Ki-*ras* codon 12 by PCR may distinguish pancreatic carcinoma from other periampullary carcinomas that have better prognoses.

NEW TECHNIQUES HAVE become available to diagnose pancreatic and other periampullary carcinomas. The range of diagnostic techniques has expanded to include computed tomography (CT) scanning, ultrasonography, endoscopic cholangiopancreatography, angiography, transhepatic cholangiography, and the measurement of tumor markers in the serum. This sudden burst of new technology has not yielded a precise method for distinguishing pancreatic cancer from other periampullary carcinomas, however. Even the microscopic distinction between pancreatic and other periampullary carcinomas can be extremely difficult in patients with massive tumors.

Pancreatic carcinoma is the fourth leading cause of cancer death among men in the United States, Europe, and Japan. Most patients with symptoms of pancreatic cancer already have unresectable, incurable disease. Even when the cancer is confined to the pancreas at the time of surgery, the 5-year survival rate in most series does not exceed 5%.[1] Conversely ampullary carcinomas should be treated by Whipple procedure, which can cure a significant number of patients.[2,3] Thus it is important to distinguish pancreatic carcinoma from other periampullary tumors that have better prognoses.

Point mutation leading to the activation of *ras* genes is a well-studied mechanism for converting proto-oncogenes into transforming sequences. Activating mutations at codons 12, 13, or 61 have been detected in a variety of human neoplasms with variable frequencies.[4,5] By far the highest incidence was recently observed in pancreatic carcinomas, in which about 90% show Ki-*ras* gene mutations.[6,7] These reports prompted us to study in detail, by polymerase chain reaction (PCR),[8] the mutations of this gene for the purpose of distinguishing pancreatic from other periampullary carcinomas. The very high frequency of the Ki-*ras* codon 12 mutation in pancreatic carcinoma is presented in this paper, and it is suggested that this mutation may allow precise distinction between pancreatic and other periampullary carcinomas.

Materials and Methods

Patients

We selected a total of 105 periampullary cancer specimens from patients treated in the Second Department

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of Surgery of Nagasaki University between 1975 and 1989. Of these specimens, 40 were pancreatic carcinomas, 48 were bile duct carcinomas (19 distal, 6 middle, and 23 proximal), 16 were ampullary carcinomas, and one was duodenal carcinoma.

The study is based on patients from whom tumor tissues were still available for molecular genetic analysis and for whom complete clinical follow-up had been documented. All of these patients had undergone surgical resection. The operations performed were the Whipple procedure for 66 patients with periampullary and middle bile duct carcinoma, total pancreatectomy for 11 patients with pancreatic carcinoma, distal pancreatectomy for 5 patients with distal pancreatic carcinoma, and bile duct resection for 13 patients, and bile duct resection with combined hepatic resection for 10 patients with proximal bile duct carcinoma. The latter group was chosen as the control for comparison with distal bile duct carcinoma.

Tumor Materials

Formalin-fixed paraffin-embedded tumor samples from 105 patients were available. A control study was conducted on pancreatic tissues free of cancer that were embedded in separate paraffin blocks from 10 patients receiving total pancreatectomy. A section of each paraffin block was stained with hematoxylin and eosin and examined for the presence of tumor cells. Five adjacent 10-μm sections were taken and used for analyzing point mutations in the Ki-*ras* genes by PCR.

Synthetic Oligonucleotides

The oligonucleotides were synthesized by a DNA synthesizer (Applied Biosystems, Foster City, CA). Sequences of oligonucleotide primers for PCR and oligonucleotide probes for detection of Ki-*ras* mutations were as described by Verlaan-de Vries et al.[9]

DNA Amplification

One 10-μm section was used for each PCR amplification. The method of amplification followed essentially that described by Shibata et al.[10] After deparaffination, the tissue was suspended in 100 μL H_20 and incubated for 10 minutes at 100 C. The reaction mixture contained tissue pellets, 0.6 μg of each primer, 800 μmol/L deoxynucleotide triphosphates, 16.6 mmol/L $(NH_4)_2$ SO_4, 67 mmol/L TRIS-HCl, 6.7 mmol/L $MgCl_2$, 10 mmol/L 2-mercaptoethanol, 200 μg/mL buffer solution (BSA), and 2 units of Taq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT) in a total volume of 100 μL. The mixture was incubated at 95 C for 60 seconds to denature the DNA and allowed to anneal at 48 to 55 C for 90 seconds. Primer extension was performed at 72 C for 90 seconds. Forty cycles of denaturation, hybridization, and extension of DNA were repeated by an automated heatblock (Program Temp Control System, Astec Inc., Fukuoka).

Oligomer Hybridization

The method used was described by Verlaan-de Vrice et al.[9] Amplified DNA was spotted onto nylon filters (Gene Screen Plus, New England Nuclear, Boston, MA). The filters were dried and prehybridized for 3 to 6 hours at 56 C in 3.0 mol/L tetramethylammonium chloride, 50 mmol/l TRIS-HCl, 2 mmol/L ethylenediaminetetraacetic acid (EDTA), 100 μg/mL sonicated and denatured salmon sperm DNA, 0.1% sodium dodecyl sulfate (SDS), and 5 × Denhardt's solution, and hybridized for 1 hour at 56 C in the mixture containing ^{32}P-labeled oligomer probes. After hybridization the filters were washed twice in 2 × standard saline citrate (SSC), 0.1% SDS for 5 minutes at room temperature, and once in 5 × SSC, 0.1% SDS for 10 minutes at 60 C. Subsequently the filters were incubated for 1 hour in the hybridization buffer without Denhardt's solution and salmon sperm DNA. The filters were exposed to Kodak XSR film for 12 hours at −70 C.

Statistic Analysis

The chi square test was used to determine statistical significance in the frequency of the mutations between the periampullary carcinoma. Cumulative survival rates and curves were calculated using the Kaplan-Meier method. The generalized Wilcoxon test was used to compare survival curves. P values less than 0.05 were considered to be statistically significant.

Results

Autoradiograms of the Hybridization

By PCR, we amplified Ki-*ras* sequences successfully from the paraffin-embedded tumor samples. Autoradiographs of identical dot blots demonstrate convincingly hybridizations to Ki-*ras* codon 12 in all specimens (Fig. 1). By selective hybridization to mutation-specific oligomers, we identified Ki-*ras* mutations at codon 12 in 50 of the 105 tumors resulting in substitution of aspartic acid (GAT), arginine (CGT), valine (GTT), alanine (GCT), or cysteine (TGT) for the normal amino acid glycine (GGT). This result was confirmed in duplicate. In routine analyses we were able to detect a point mutation when tumor cells were present in at least 10% of the cells in the sample. We detected mutations of Ki-*ras* codon 12 in 35 of 40 cases of pancreatic carcinoma, 3 of 16 ampullary carcinomas, 9 of 19 distal bile duct, 0 of 6 middle bile duct, 2 of 23 proximal bile duct carcinomas, and one duodenal cancer diagnosed on the basis of histopathology (Table 1).

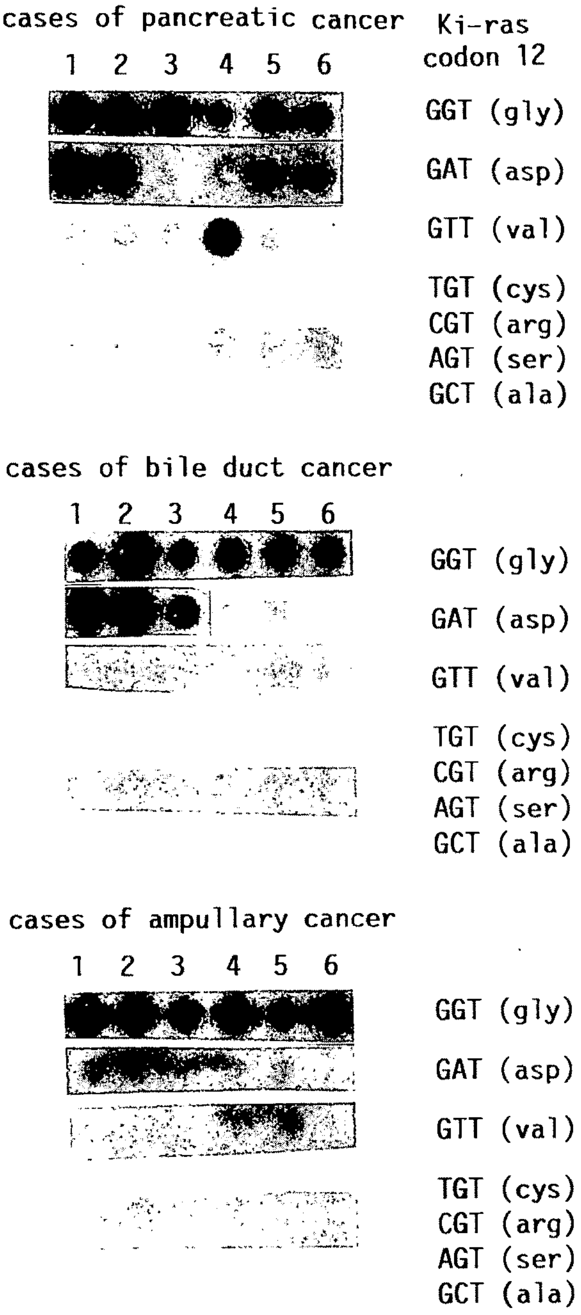

FIG. 1. Detection of point mutations at codon 12 of the Ki-ras gene by dot blot hybridization. Autoradiograms of hybridizations in identical dot blots by oligonucleotide probes for the normal sequence GGT coding glycine of codon 12 are shown in all cases because of contamination by normal tissue. In patients 1, 2, 5, and 6, who had pancreatic carcinoma, a point mutation of GGT to GAT was detected. A GGT to GTT mutation was found in patient 4, and a mutation was not detected in patient 3. In six isolates of bile duct carcinoma, patients 1, 2, and 3 showed GGT to GAT mutation. No mutation was detected in these ampullary carcinomas.

TABLE 1. *Incidence of Mutations in Ki-*ras *Codon 12 According to Histopathologic Diagnosis*

Histopathologic Diagnosis	Incidence
Pancreatic carcinoma	35/40 (87.5%)
Ampullary carcinoma	3/16 (18.8%)*
Duodenal cancer	1/1 (100%)
Bile duct carcinoma	11/48 (22.9%)†
Distal	9/19 (47.4%)
Middle	0/6 (0%)
Proximal	2/23 (8.7%)

* $p < 0.005$ *vs.* pancreatic carcinoma.
† $p < 0.005$ *vs.* pancreatic carcinoma.

Analysis of 40 Pancreatic Carcinoma Isolates

Of the 40 samples, 35 (87.5%) showed a clearly specific signal indicating that the corresponding mutation was present in the pancreatic carcinoma isolate. Noncancerous pancreatic tissues were negative for Ki-*ras* mutations. One specimen exhibited two different Ki-*ras* mutations simultaneously in the same tumor. In 24 of the 35, the mutation was from glycine (GGT) to aspartic acid (GAT), 7 were mutated to valine (GTT), 4 to arginine (CGT), and one to cysteine (TGT) (Table 2). Finally five samples with sufficient pancreatic tumor cells did not contain a mutation in codon 12 of the Ki-*ras* gene. Of these five, two tumors were tubular adenocarcinoma in histologic type. In these cases, preoperative serum CA19-9 high levels and the poor outcomes were consistent clinically with a diagnosis of pancreatic carcinoma. The remaining two cases were diagnosed histologically as mucinous carcinoma or papillary adenocarcinomas with mucin production. One tumor diagnosed as cribriform carcinoma was a rare type of histopathology (Table 3). All of these five isolates were confirmed on histopathologic review to be the pancreatic cancer.

Analysis of 16 Ampullary Carcinoma Isolates

Three isolates of the ampulla of Vater carcinomas had mutations in codon 12 of Ki-*ras*. In one of these ampullary carcinomas, the tumor was confined to the ampulla of Vater. In the other two cases, microscopically massive tumors in the pancreatic tissue was evident. One of these two cases was thought to have been misdiagnosed histopathologically and should be considered as pancreatic carcinoma. This was because the ampulla of Vater epithelium was not replaced completely by tumor cells arising from the 5 cm diameter tumor in the pancreas head. In

TABLE 2. *Ki-*ras *Codon 12 Mutation in 40 Isolates of Pancreatic Carcinoma*

	Codon 12	Amino Acid	Incidence
Wild type	GGT	Glycine	5/40 (12.5%)
Mutation	GAT	Aspartic acid	24/40 (60.0%)*
	GTT	Valine	7/40 (17.5%)*
	CGT	Arginine	4/40 (10.0%)
	TGT	Cysteine	1/40 (2.5%)

* One tumor showed two different mutations.

TABLE 3. *Patients With Pancreatic Carcinoma Without a Ki-*ras *Codon 12 Mutation*

Patient Age	Sex	Histologic Diagnosis	Mucin Production	Serum CA-19-9 Value	Outcome
77	M	Tubular	–	71	Dead 19 mo
63	M	Tubular	–	609	Dead 4 mo
67	F	Papillary	++	—	Dead 99 mo
68	F	Cribriform	–	—	Operative death
74	F	Mucinous	+++	1.6*	Alive 36 mo

* Lewis blood type (a–, b–).

the other case, it was difficult to revise the diagnosis to pancreatic carcinoma by histologic review because there were two manifest cancer lesions in both the pancreas head and the ampulla of Vàter (Table 4).

Analysis of One Duodenal Cancer Isolate

One case of duodenal cancer by histologic diagnosis had a mutation in codon 12 of Ki-*ras*. This patient had obstructive jaundice and stenosis of the second portion of the duodenum. The preoperative level of CA19-9 in serum was within the normal range because of the Lewis blood type (a–, b–). A histopathologic review of this tumor demonstrated cancer lesions in the pancreas head and in the wall of the duodenum. Although the tumor had been diagnosed histologically as poorly differentiated adenocarcinoma with the giant cells, pleomorphic carcinoma of the giant cell type that is found uncommonly in the pancreatic carcinoma may be correct. After this review the diagnosis in this case was revised to pancreatic cancer (Table 4).

Analysis of 48 Bile Duct Carcinomas Isolates

No mutation was identified in samples from six patients with middle bile duct carcinoma. Two of twenty-three cases of proximal bile duct carcinoma selected as controls for the distal bile duct carcinoma had point mutations in codon 12 of Ki-*ras*. The frequency of mutations in proximal bile duct carcinoma was 8.7%. Conversely 9 (47.4%) of 19 isolates of distal bile duct carcinoma had the mutations (Table 5). There was a large difference in mutation prevalence between distal and upper bile duct carcinomas. These results compelled us to review the histopathology of the nine specimens with the mutations. Surprisingly the review uncovered two cases of pancreatic cancer in the nine cases of distal bile duct carcinoma with the mutations. Neither of the two cases misdiagnosed as distal bile duct carcinoma had cancer cells in the epithelium of their common bile ducts, even though they were compressed by the pancreatic cancer. Except for one case of early stage carcinoma confined to the fibromuscular layer of the common bile duct, it was difficult to reconsider the pathologic diagnosis for the other six cases with the mutations by review of histology.

Finally we could revise the histopathologic diagnosis of four patients from other periampullary carcinoma to pancreatic carcinoma. In all these cases, the final diagnosis was based on histopathologic reviews, but not on the presence of the Ki-*ras* mutations, although an opportunity for the further histologic review was offered by the detection of the Ki-*ras* mutations.

Incidences of Mutations After Revision of Histopathologic Diagnoses

The prevalance of Ki-*ras* codon 12 mutations according to revised pathologic diagnoses are summarized in Table 6. The previous histopathologic diagnosis was unchanged in two cases of ampullary carcinoma and in seven of distal bile duct carcinoma. On the basis of the new pathologic diagnoses, the prevalence of point mutations in Ki-*ras* codon 12 was 2 (13.3%) of 15 in ampullary carcinoma, 39 (88.6%) of 44 in pancreatic cancer, and 7 (41.2%) of 17 cases of distal bile duct carcinoma. Although the high incidence of point mutations was seen in the distal bile duct carcinoma even after diagnoses were revised, the prevalence of the mutations in total cases of distal, middle, and proximal bile duct carcinoma was 19.6% (Table 6).

Cumulative Survival Rates Based on Revised Diagnosis

At the end of February 1991, the cumulative survival rates based on revised diagnosis were as illustrated in Fig-

TABLE 4. *Patients With Ampullary and Duodenal Carcinoma With the Ki-*ras *Codon 12 Mutation*

Patient Age	Sex	Type of Mutation	Pancreas Invasion	Duodenal Invasion	Serum CA-19-9	Outcome
Ampullary carcinoma						
70	M	TGT (cys)	–	–	28	Dead 48 mo, liver metastases
66	F	GAT (asp)	++	++	22	Dead 6 mo, liver metastases
79	M*	GAT (asp)	++	++	—	Dead 6 mo, liver metastases
Duodenal cancer						
67	M*	GTT (val)	+++	+++	10†	Dead 8 mo, lymph node recurrence

* Histopathologic diagnosis was revised to pancreatic cancer. † Lewis blood type (a–, b–).

TABLE 5. *Patients With Distal Bile Duct Carcinoma With the Ki-*ras *Codon 12 Mutation*

Patient Age	Sex	Type of Mutation	Pancreas Invasion	Duodenal Invasion	Serum CA-19-9	Outcome
53	M	AGT(ser)	–	–	12	Alive 30 mo
64	M	GAT(asp)	+++	+		Dead 12 mo, cardiac infarction
70	M*	GAT(asp)	+++	–	66	Dead 2 mo, pneumonia
57	M	GAT(asp)	+++	+++	63	Dead 17 mo, lung metastases
74	F	CGT(arg)	+++	++	17	Dead 14 mo, lung metastases
81	F*	CGT(arg)	++	++	8†	Alive 10 mo
69	F	GTT(val)	++	+++	90	Dead 16 mo, liver metastases
48	M	GTT(val)	+++	++	10†	Alive 24 mo
63	F	GTT(val)	++	+++	11†	Dead 20 mo, liver metastases

* Histopathologic diagnosis was revised to pancreatic cancer.

† Lewis blood type (a–, b–).

ure 2. Ten patients who died from a nonmalignant process such as operative death, pneumonia, intracerebral bleeding, and hypoglycemia after total pancreatectomy were excluded from this study. The 5-year survival rates of 13 patients with ampullary carcinoma, 42 with bile duct carcinoma, and 40 with pancreatic carcinoma were 32%, 30%, and 12%, respectively. By the generalized Wilcoxon test, there were statistically significant differences in the survival curves for patients with ampullary carcinoma and for those with pancreatic carcinoma ($p < 0.05$), and between patients with bile duct carcinoma and those with pancreatic carcinoma ($p < 0.01$) (Fig. 2).

Discussion

By taking advantage of the PCR *in vitro* gene amplification technique to increase sensitivity, we have shown that mutations in the Ki-*ras* gene can be detected readily in a tissue specimen as small as that contained in a single 10-μm paraffin section. The ability to use formalin-fixed paraffin-embedded tissue specimens stored at room temperature for many years increases greatly the potential for applications of the methods. Thus PCR has made it possible to analyze retrospectively the frequency and character of mutant oncogenes in various tumor types. By far the highest incidence has been observed in pancreatic carcinoma, showing Ki-*ras* codon 12 mutations in 90%.[6,7] Our findings also showed that most carcinomas of the exocrine pancreas contain activated Ki-*ras* oncogenes. These results imply that the mutation of the Ki-*ras* gene could serve as a diagnostic molecular genetic marker for this cancer. A more remarkable observation is that the mutations in all mutation-positive pancreatic carcinomas reported thus far are restricted to Ki-*ras* codon 12.[6,7,11] This specificity and prevalence of Ki-*ras* codon 12 mutations in pancreatic cancer prompted us to suggest use of this genetic maker in distinguishing pancreatic from periampullary carcinoma.

TABLE 6. *Prevalence of Mutation in Ki-*ras *Codon 12 in the Patients Based on the Revised Diagnosis*

Revised Diagnosis	Incidence
Pancreatic carcinoma	39/44 (88.6%)
Ampullary carcinoma	2/15 (13.3%)*
Bile duct carcinoma	9/46 (19.6%)†
Distal	7/17 (41.2%)
Middle	0/6 (0%)
Proximal	2/23 (8.7%)

* $p < 0.005$ *vs.* pancreatic carcinoma.

† $p < 0.005$ *vs.* pancreatic carcinoma.

Adenocarcinomas of the periampullary region originate in the ampulla, distal bile duct, duodenum, or head of the pancreas. Although the modes of presentation and treatment options for these tumors are similar, their prognoses are quite different, with that for adenocarcinoma of the head of the pancreas being much worse than that for the other periampullary tumors. Differential diagnosis of periampullary carcinomas is difficult, however, especially in advanced cases. We introduced PCR as a new diagnostic technique into the differential diagnosis of pancreatic carcinoma from periampullary carcinoma. Three samples of ampullary carcinoma, nine of distal bile duct carcinoma, and one of duodenal cancer contained

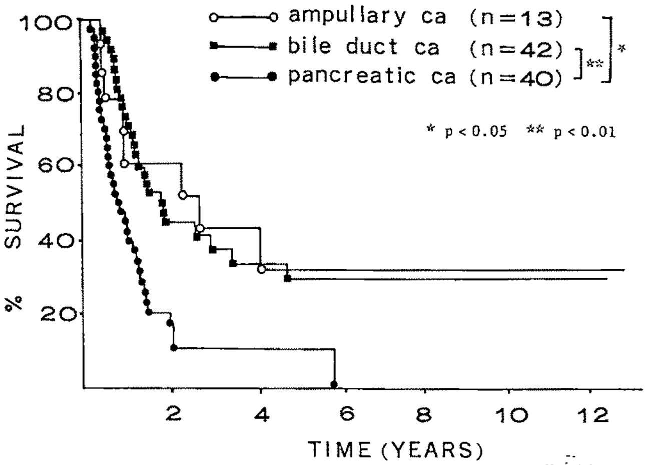

FIG. 2. Cumulative survival rates after surgical resection for patients with adenocarcinoma of the ampulla of Vater, bile duct, and pancreas.

Ki-*ras* codon 12 mutations. Two of these cases were of early stage carcinoma in the common bile duct and ampulla of Vater. By these cases we learned that the ampullary and bile duct carcinomas can express mutation in Ki-*ras* codon 12. The prevalence of such a point mutation in the ampullary and upper bile duct carcinomas, however, was less than 15%. In overall carcinomas of bile duct, the incidence of the mutations was within 20%. These results indicated that pancreatic carcinoma could be distinguished from carcinomas of the ampulla of Vater and bile duct.

By contrast the frequency of the mutations in distal bile duct carcinoma was 47.4%. This high frequency contrasted with that of upper bile duct carcinomas even though both originated from the biliary tract. With regard to this contrast, two reasonable suppositions can be made. First of all distal bile duct carcinoma could include cancers of pancreatic head. Secondly because there are long-term survivors of proximal bile duct carcinoma—the so called Klatskin tumor—with T-tube external or internal drainage procedures,[12] the slow progression of this disease may represent a carcinogenesis different from distal bile duct carcinoma. This requires further investigation.

The *in vitro* PCR gene amplification technique for detecting point mutation enhances diagnostic sensitivity more than does the RNAase A mismatch cleavage method.[7] We have shown that mutations in the Ki-*ras* gene are detectable in a tissue specimen of a single 10-μm paraffin section. Endoscopic biopsy from the duodenal wall or ampulla of Vater and pericutaneous aspiration biopsy from the periampullary area under the guide of ultrasonography are safely possible to collect small samples of tumor tissues. These small tissue samples would be sufficient to detect the mutations by PCR. Preoperative analysis of the mutations could be applied to distingish pancreatic from other periampullary carcinomas having better prognoses. By distinguishing preoperatively between pancreatic and other periampullary carcinoma, both surgical and medical therapy can be optimized.

References

1. Gudjonsson B. Cancer of the pancreas: 50 years of surgery. Cancer 1987; 60:2284–2303.
2. Kellum MJ, Clark J Miller HH. Pancreatoduodenectomy for resectable malignant periampullary tumors. Surg Gynecol Obstet 1983; 157:362–366.
3. Hayes DH, Bolton JS, Willis GW, et al. Carcinoma of the ampulla of Vater. Ann Surg 1987; 206:572–577.
4. Bos LJ, Fearon RE, Hamilton RS, et al. Prevalence of ras gene mutation in human colorectal cancers. Nature 1987; 327:293–297.
5. Lemoine RN, Mayall SE, Wylie SF, et al. High frequency of ras oncogene activation in all stages of human thyroid tumorigenesis. Oncogene 1989; 4:159–164.
6. Smit VTHBM, Boot AJM, Fleuren CJ, et al. KRAS codon 12 mutations occur very frequently in pancreatic adenocarcinomas. Nucleic Acids Research 1988; 10:7773–7782.
7. Almoguera C, Shibata D, Forrester K, et al. Most human carcinomas of the exocrine pancreas contain mutation c-K-ras genes. Cell 1988; 53:549–554.
8. Saiki RK, Scharp S, Faloona F, et al. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 1985; 230:1350–1354.
9. Verlaan-de Vries M, Bogaard ME, Van den Elst H, et al. A dotblot screening procedure for mutated ras oncogenes using synthetic oligodeoxynucleotides. Gene 1986; 50:313–320.
10. Shibata DK, Arnheim N, Martin W. Detection of human papilloma virus in paraffin-embedded tissue using the polymerase chain reaction. J Exp Med 1988; 167:225–230.
11. Grunewald K, Lyons J, Frohich A, et al. High frequency of Ki-ras codon 12 mutation in pancreatic adenocarcinomas. Int J Cancer 1989; 43:1037–1041.
12. Klatskin G. Adenocarcinoma of the hepatic duct at its bifurcation within the porta hepatis. Am J Surg 1965; 38:241–256.

Pancreatic Resection *Versus* Peritoneal Lavage in Acute Necrotizing Pancreatitis

A Prospective Randomized Trial

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Twenty-one patients with acute fulminant alcoholic pancreatitis were randomly allocated to either pancreatic resection group (11 patients) or nonoperative peritoneal lavage group (10 patients). Only patients under 50 years were included in the study to minimize the role of other severe disease. These patients represented the most severe cases of acute pancreatitis at our Department, constituting only 2% of all patients with acute pancreatitis during this period. The diagnosis was based on clinical symptoms and on signs indicating severely impaired systemic organ functions. All patients underwent contrast-enhanced computed tomography (CT), which showed contrast enhancement below 30 Hounsfield units. In the operated cases, the diagnosis of necrotizing pancreatitis was verified histologically. All patients with conservative treatment had dark brown fluid at peritoneal puncture. There was a difference (nonsignificant) in mortality (3/11 and 1/10, respectively), complication rate, or in the need of reoperations between the groups. Nonoperative peritoneal lavage was followed with shorter treatment at the intensive care unit (16.2 *versus* 25.9 days, respectively,). The hospital stay also was significantly shorter in the nonoperative group (44.3 *versus* 56.1 days). The results indicate that intensive conservative treatment is justified as an initial therapy even in the most severe cases of acute pancreatitis.

ACUTE FULMINANT PANCREATITIS is the most severe form of the disease, with a life-threatening course.[1] The management of these patients is still controversial, because no medical treatment has been shown to be beneficial. The role of peritoneal lavage remains debatable,[2-7] and there are no controlled studies to compare intensive conservative and operative treatment. To perform any controlled studies between conservative and operative treatment, reliable diagnostic methods and classification of the severity of the disease are needed. There are several problems involved with multicenter studies, because of variation in cause, treatment protocol, and therapeutic methods that may lead to different results, not necessarily depending on the factors studied. Conversely the number of patients in one unit may be too small for any far-going conclusions. We have performed a single-center prospective randomized study to compare nonoperative peritoneal lavage and early pancreatic resection as the primary treatment for acute fulminant pancreatitis. Only the most severe cases of acute pancreatitis were included in the study and the diagnosis was based on an early fulminant clinical course, contrast-enhanced CT,[8,9] and histologic examination in the operated cases.

Materials and Methods

Twenty-one consecutive patients treated at the Second Department of Surgery, Helsinki University Hospital, between May 24, 1984 and September 16, 1988, were included in the study. All patients filled the entry criteria of fulminant acute pancreatitis. During the study period, a total of 948 patients with acute or acute relapsing pancreatitis were treated at the Department. Blood samples were for the estimation of the modified prognostic signs,[10] and C-reactive protein (CRP) values were taken daily.

Entry Criteria

Patients under 50 years with acute alcoholic pancreatitis were accepted. The diagnosis was based on the clinical symptoms and laboratory findings. When severe pancreatitis was suspected, a contrast-enhanced CT was performed as follows: scanning was done with a Somatom DR2 (Siemens, 8-mm slice thickness, 5 seconds scanning time) scanner. The abdomen was first scanned without intravenous contrast agent to estimate the pancreatic and

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extrapancreatic changes and to localize the pancreas. Thereafter a rapid bolus of contrast agent (Conray 400, meglumine iothalamate 1 mL/kg, 400 mgI/kg) was injected intravenously, and the contrast enhancement of the pancreas was recorded by fast sequential exposure at a selected level of the pancreas. The contrast enhancement was measured at three different points in the pancreas and a curve was drawn by computer. Patients with more than four extrapancreatic scores (10) and the contrast enhancement below 30 Hounsfield units were considered candidates for the study. Before the disease was considered fulminant, the patients also had to show early signs of systemic organ failure, such as pulmonary insufficiency (PaO_2 below 60 mmHg), renal failure (urine output below 50 mL/hr, increasing urinary creatinine), shock, or peritonitis. Randomization was performed by the method of supernumerically sealed envelopes. The treatment was either nonoperative peritoneal lavage (n = 10) or subtotal pancreatic resection (n = 11).

Treatment

All patients had nasogastric suction and antibiotics (tobramycin and clindamycin). Fluid resuscitation was performed at the intensive care unit, and all patients had a urinary and central venous catheter and arterial cannula for repeated arterial gas analysis. The patients were intubated and connected to a ventilator when necessary. The gastric pH was kept above 5 with H_2-blockers and, when necessary, gastric installation of antacid was used to prevent stress ulceration.

Peritoneal lavage. In this group (n = 10), the patients were taken into the operating theater, where a peritoneal lavage catheter (Tenchhof) was inserted through a midline minilaparotomy incision under local anesthesia. Peritoneal lavage was started immediately with fluid (Peridialys III, Leiras Pharmaceuticals Inc., Turku, Finland) at a rate of 1000 mL/hr and continued for 8 to 12 days. No antibiotics or antiprotease drugs were added to the fluid. Complications of pancreatitis were treated operatively when necessary.

Pancreatic resection. The patients (n = 11) were operated on immediately after randomization. A midline incision was performed. The omentum was separated from the colon and the left colon was mobilized. Thereafter the splenogastric vessels were ligated and divided and the spleen mobilized. The pancreatic capsule was opened on both sides of the pancreas and the pancreas was mobilized and dissected to the right side of the portal vein, where it was divided. A choledochostomy was routinely performed, and a T-tube cholangiography was done. The abdomen, and especially the pancreatic area, were drained using a total of four intra-abdominal drains. The pancreatic bed then was flushed using Peridialys III fluid, 1000 mL/hour for 8 to 12 days. When needed the complications of the disease were treated by secondary operation.

TABLE 1. *Age and Sex Distribution of the Patients*

Patient Group	Resection	Lavage
M	10	9
F	1	1
Age (yr)	42.1	34.4
Range	26–50	27–48

TABLE 2. *Duration of Symptoms Before Randomization*

Duration (hr)	Resection	Lavage
<24	6	6
24–72	2	3
>72	3	1

Results

The sex and age distribution of the patients is shown in Table 1. Eight patients in the resection group and nine in the lavage group had their first episode of pancreatitis, and only three (resection) and one (lavage) had relapsing episodes. Four patients in the resection group and in the six patients in the lavage group were admitted from other hospitals. The duration of symptoms before randomization is shown in Table 2.

The prognostic signs and the highest CRP value within 48 hours after admission are shown in Table 3.

CT

The duration of symptoms until the first CT examination was 63.6 and 60.0 hours, respectively. Contrast-enhanced CT was done once in eight patients in the resection group and once in seven in the lavage group. In both groups three patients had pancreatitis of intermediate severity in their first CT, but the second CT, performed 3 to 6 days later, showed progression to necrotizing pancreatitis.

Mortality and Morbidity

The duration of hospital stay was 56.1 days (range, 25 to 165) in the resection group and 44.3 days (range, 15

TABLE 3. *Prognostic Signs and Highest CRP Value Within 48 Hours*

Variable	Resection	Lavage
Prognostic signs	3.6 (range, 0–7)	4.3 (range, 2–8)
CRP value	210 (range, 51–274)	289 (range, 196–320)

CRP, C-reactive protein.

TABLE 4. *Mortality and Complications*

Variable	Resection	Lavage
Mortality	3/11	1/10
Respiratory insufficiency	6/11	4/10
Renal insufficiency	4/11	1/10
Sepsis	4/11	3/10
Abscess	4/11	4/10
Other major complication	5/11	7/10

to 97) in the lavage group. Treatment at the intensive care unit was 25.9 days (range, 7 to 100) in the resection group and 16.2 days (range, 5 to 52) in the lavation group. Three patients in the resection group (27%) and one in the lavage group (10%) died. The cause of death was multiorgan failure in all cases. There were no operative deaths or severe operative complications. A complicated course of the disease was seen in eight patients after resection and in five patients with peritoneal lavage. These results are summarized in Table 4. Reoperations were performed in five resected patients and in five initially nonoperated patients. The reoperations are demonstrated in Table 5.

Discussion

The results of the treatment of acute pancreatitis vary depending of the diagnosis, classification, cause, and management of the disease. Still in most series there remains a small group, 2% to 4% of all patients, who have overwhelmingly severe pancreatitis (*i.e.*, fulminant pancreatitis) and a further 5% or more who will develop local complications (*i.e.*, complicated acute pancreatitis). The present study deals only with the most severe cases. The diagnosis was based on clinical signs of multiorgan failure, contrast-enhanced CT, histology of resected specimens in operatively treated patients, and the presence of dark intraperitoneal fluid in peritoneal puncture.

The management of these patients is still a matter of debate.[11-13] In our department we have a long experience of early pancreatic resections,[12,14] and since the onset of aggressive treatment the results have significantly improved. In a randomized study, early pancreatic resection proved to be slightly, although not significantly, better than operative peritoneal lavage in the treatment of hemorrhagic pancreatitis[13] in terms of survival. At the time of that study, some encouraging reports were given on nonoperative peritoneal lavage.[6] Based on these facts, we started a prospective, randomized trial to compare early pancreatic resection with nonoperative peritoneal lavage as the primary treatment in acute fulminant disease. Our study was already underway when Mayer et al.[7] reported their multicenter trial comparing nonoperative lavage with conservative treatment without lavage. Their study did not show any benefits of lavage. Conversely it did not have a negative influence on the results. Yet we still believe that a larger single-center study might give some new data in this respect, because we have found that tapping of the hemorrhagic peritoneal fluid often improves respiratory function, and peritoneal lavage may postpone or even dispense with the need for peritoneal or hemodialysis in renal insufficiency. In the present series, late complications were operatively treated when necessary. Five patients in the resection group and four patients in the lavage group were reoperated for necrosis or abscess in the peripancreatic region. Thus early pancreatic resection did not prevent the development of necrosis in the remaining pancreas or in the peripancreatic area. Further resection significantly prolonged the need of treatment at the intensive care unit and hospital stay. Major complications developed at an equal rate in both groups, but the mortality rate was smaller in the lavage group, 3 of 11 and 1 of 10, respectively. The cause of death was multiorgan failure in all patients. There were no operative complications. Postoperatively one patient developed a pancreatic fistula, which was operatively treated by a small pancreatic re-resection and canalization of the area. The number of patients in the present study is small because only the most severe cases of alcoholic pancreatitis with

TABLE 5. *Reoperations in Both Groups*

Resection			Lavage		
Patient	Cause	No. of Operations	Patient	Cause	No. of Operations
1	Abscess	3	1	Chylothorax pericardiae effusion	2
2	Hematoma and necrosis	2	2	Bleeding ulcer, abscess	3
3	Exploration	1	3	Abscess, colocutaneous fistula	2
4	Abscess, tracheostomy, revision of decubitus, evacuation of hematoma, repair of Virsungian fistula and ventral hernia, neurolysis (ulnar paresis)	6	4	Abscess	3
			5	Abscess, tracheostomy, pyelostomy, closure of colocutaneous fistula, subtotal pancreatectomy	5
5	Abscess, choldochal fistula	2			
Total		14			15

the worst prognosis were included and the including criteria were very strict. We finished the study when we believed that conservative treatment was better, September 9, 1988.

The present results are in agreement with the earlier reports[15] that recommend an initial expectation and interim conservative management, with later surgical intervention when necessary for debridement of necrotic mass or treatment of other complications. We have not performed aspirations of the peripancreatic area for bacterial cultures, as suggested by Beger et al.,[16] but this probably gives further information for adequate timing of surgical intervention.

It is likely that improved results during the past years are basically due to improved initial intensive care, including respiratory support, treatment of renal insufficiency, improved treatment of sepsis, and adequate early fluid resuscitation.[17]

Acknowledgments

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References

1. Glazer G. Contentious Issues in acute pancreatitis. *In* Glazer G, Ranson JHC, eds. Acute Pancreatitis: Experimental and Clinical Aspects of Pathogenesis and Management. London: Bailliére Tindall, 1988, pp 1–36.
2. Gjessing J. Peritoneal dialysis in severe acute hemorrhagic pancreatitis. Acta Chir Scand 1967; 133:645–647.
3. Ranson JHC, Spencer FC. The role of peritoneal lavage in severe acute pancreatitis. Ann Surg 1978; 187:565–575.
4. Stone HH, Fabian TC. Peritoneal dialysis in the treatment of acute alcoholic pancreatitis. Surg Gynecol Obstet 1980; 150:878–882.
5. Balldin G, Ohlsson K. Demonstration of pancreatic-antiprotease complexes in the peritoneal fluid of patients with acute pancreatitis. Surgery 1979; 85:451–456.
6. Lasson A, Balldin G, Genell S, et al. Peritoneal lavage in severe acute pancreatitis. Acta Chir Scand 1984; 150:479–484.
7. Mayer AD, McMahon MJ, Corfield AP, et al. Controlled clinical trial of peritoneal lavage for the treatment of severe acute pancreatitis. N Engl J Med 1985; 312:399–404.
8. Kivisaari L, Somer K, Standertskjöld-Nordenstam C–G, et al. Early detection of acute fulminant pancreatitis by contrast-enhanced computed tomography. Scand J Gastroenterol 1983; 18:39–41.
9. Kivisaari L, Somer K, Standertskjöld-Nordenstam C–G, et al. A new method for the diagnosis of acute hemorrhagic necrotizing pancreatitis using contrast-enhanced CT. Gastrointest Radiol 1984; 9:27–30.
10. Schröder T, Kivisaari L, Somer K, et al. Significance of extra-pancreatic findings in computed tomography (CT) in acute pancreatitis. Eur J Radiol 1985; 5:273–275.
11. Bradley EL, Fulenwider JT. Open treatment of pancreatic abscess. Surg Gynecol Obstet 1984; 159:509–513.
12. Davidson ED, Bradley EL. Marsupialization in the treatment of pancreatic abscess. Surgery 1984; 89:252–256.
13. Kivilaakso E, Lempinen M, Mäkeläinen A, et al. Pancreatic versus peritoneal lavation for acute pancreatitis. A randomized prospective study. Ann Surg 1984; 199:426–431.
14. Kivilaakso E, Fräki O, Lempinen M. Resection of the pancreas for acute fulminant pancreatitis. Surg Gynecol Obvstet 1981; 152: 493–498.
15. Beger HG, Krautzberger W, Bittner R, et al. Results of surgical treatment of necrotizing pancreatitis. World J Surg 1985; 9:972–979.
16. Beger HG, Bittner R, Block S, et al. Bacterial contamination of pancreatic necrosis. A prospective clinical study. Gastroenterology 1986; 91:433–438.
17. Lehtola A, Kivilaakso E, Puolakkainen P, et al. Effects of dextran 70 versus crystalloids in the microcirculation of porcine hemorrhagic pancreatitis. Surg Gynaecol Obstet 1986; 162:556–562.

Sex Differences in Gallstone Pancreatitis

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From a computerized database comprising 28 pertinent items in each of a consecutive series of 664 patients with cholelithiasis, differences were studied between men and women. In 52 patients there was a documented attack of acute pancreatitis (7.8%). Twenty-five of 174 men had pancreatitis, compared with 27 of 490 women ($p < 0.0001$). Men developed gallstones later in life than women, but suffered gallstone pancreatitis earlier in life and in the course of their gallstone-related disease. A history of flatulent dyspepsia, chronic cholecystitis, and biliary colic was less common in men than in women with pancreatitis ($p < 0.0001$). Men with pancreatitis had fewer stones in their gallbladders than did women ($p = 0.0002$). The cystic duct and the common bile duct in the pancreatitic patient were more likely to be dilated ($p < 0.0001$). In the nonpancreatitic group, these ducts were larger in men. Pancreatic duct reflux on operative cholangiography was more common both in patients with pancreatitis 62% cf 14% ($p < 0.0001$), and in men ($p < 0.001$). Predisposition to pancreatitis relates to duct size rather than stone size per se. Men are more susceptible to gallstone migration at an early stage of their disease. In addition they have a larger diameter duct system and possibly a different anatomic disposition of the sphincter of Oddi, which predisposes them to a higher incidence of pancreatitis than women. The data suggest that it is cystic duct size that is critical in the pathogenesis of gallstone pancreatitis.

GALLSTONES ARE THE most common causative factor in acute pancreatitis in the Western world.[1,2] Although it is now established that the onset of gallstone pancreatitis coincides with gallstone migration and that small stones are usually responsible, the factors that predispose to the migration of gallstones remain ill understood, and the exact pathogenesis remains an enigma. Differences exist between the sexes in their predisposition toward gallstone pancreatitis.[3] Men and women have been further studied separately to see if additional factors, of pathogenetic significance in this condition, may emerge.

Patients and Methods

A consecutive series of 664 patients undergoing biliary tract surgery for gallstones and their complications has been studied. All the collected data were stored on a computer in the Department of Computation at the Manchester University Medical School. Twenty-eight items of information were recorded on each patient (Table 1), and emphasis was placed on differences that could be identified between the two sexes. Obtaining a satisfactory operative cholangiogram was a prerequisite to data storage. Cholangiography was performed with the patient supine by injecting 3, 8, and 16 mL 25% Hypaque (Winthrop Pharmaceuticals, New York, NY) and taking three films accordingly. Injection pressures were not measured, but the same injection technique was employed throughout the study in both sexes and in those with and without pancreatitis. If cannulation of the cystic duct proved unsuccessful, direct needle puncture of the common bile duct was employed. Approximately 10% of patients had been previously excluded because of failure to obtain a technically satisfactory operative cholangiogram. This was either as a result of extravasation of dye, poor positioning of the patient, or poor film penetration. After exclusion of these, the data was complete with the possible exception of operative findings relating to the pancreas, which are often subjective and difficult to interpret, and not always recorded even in those with a history of previous pancreatitis.

Patients in the pancreatitis group had a documented attack of acute pancreatitis with a serum amylase recorded in excess of 1000 IU during the course of their disease. The number of gallbladder stones was categorized by the exact integer up to 10, but patients with more numerous stones were merely categorized as having multiple stones.

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TABLE 1. *Variables Assessed in the Initial Database*

- Age
- Sex
- Length of history
- Presentation
  - Biliary dyspepsia
  - Acute cholecystitis
  - Chronic cholecystitis
  - Biliary colic
  - Pancreatitis
  - Back pain
  - Jaundice
  - Cholangitis
- Oral cholecystogram or ultrasound scan
  - No. of stones
  - Wall thickened
  - Nonfunction
- Gallbladder at operation
  - No. of stones
  - Wall thickened
  - Empyema (mucocoele)
  - Mucosal disease
- Findings on operative cholangiography
  - Duct filling
  - Duct diameter
  - Presence of stones
  - Common channel
  - Pancreatic duct reflux
  - Duodenal filling
- Operative measurements
  - Common bile duct
    - Dilated (>10 mm)
    - Stones palpable
  - Pancreas
    - Normal
    - Abnormal

The range of gallstone size in each individual case was recorded, as was the number of common bile duct stones, from both the operative cholangiogram and the exploratory findings.

The timing of surgery in relation to an attack of acute pancreatitis varied over the duration of the study from deferred surgery early in the study, to early surgery in the most recent cases. There were 174 men and 490 women (1:2.8). No difference existed regarding the attitude to surgical treatment of the two sexes.

Results

Of the 664 patients, 52 had pancreatitis (7.8%). The range of the length of patients' histories attributable to gallstones was from 1 week to 25 years, with a median of 1 year. Although gallstones occurred in three times as many women as men, acute pancreatitis was a significantly more common complication of gallstones in men ($p < 0.0001$) than in women (Table 2). Women underwent surgery for uncomplicated gallstones at a mean age of 48.8 years (Table 3). Although women developed pancreatitis when 6.7 years older than the average age at which they underwent elective cholecystectomy, men had surgery for gallstone pancreatitis 4 years earlier than those who underwent cholecystectomy for uncomplicated disease, and also 4 years earlier than women with pancreatitis. Thus not only are men more prone to pancreatitis, but they suffer this complication earlier in the course of their gallstone disease than do women. There was no significant difference overall in the length of history of gallstone-related symptoms between the pancreatitis and nonpancreatitis groups.

A previous history of biliary dyspepsia was much less frequent in men and women with pancreatitis than in those without this complication ($p < 0.0001$) (Table 4). Biliary colic had occurred much more frequently in both men and women who presented with no history of pancreatitis, this was particularly the case in men. A history of chronic cholecystitis was present in only 4% of men, compared with 35% of women with pancreatitis; in the

TABLE 2. *Incidence of Pancreatitis by Sex*

	M	F
No pancreatitis	149	463
Pancreatitis	25	27

Chi square = 12.8; $p < 0.0001$.

TABLE 3. *Age at Cholecystectomy*

Group	Sex	Diagnosis	Mean Age (yr)	SD (yr)	Median Age (yr)	n
1	M	No pancreatitis	55.7	13.8	58	149
2	M	Pancreatitis	51.8	14.6	51	25
3	F	No pancreatitis	48.8	15.1	50	463
4	F	Pancreatitis	55.5	17.5	63	27

Differences: group 1 *vs.* group, $p = 0.2$; group 3 *vs.* group 4, $p = 0.02$, $z = -2.4$; group 2 *vs.* group 4, $p = 0.3$; group 1 *vs.* group 3, $p = 0.001$, $z = -5.1$. Mann-Whitney U test.

TABLE 4. *Symptom Complexes at Time of Presentation*

	M		F	
Presenting Features	No Pancreatitis	Pancreatitis	No Pancreatitis	Pancreatitis
Biliary dyspepsia				
No dyspepsia	85	25	269	22
Dyspepsia	64	0	193	5
Chi square	15.2		4.8	
p	<0.0001		0.028	
Biliary colic				
No colic	60	22	168	17
Colic	88	3	295	10
Chi square	17.5		6.6	
p	<0.0001		0.01	
Chronic cholecystitis				
No cholecystitis	88	24	270	20
Cholecystitis	59	1	189	7
Chi square	16.8		5.4	
p	<0.0001		0.02	

TABLE 5. *Number of Gallstones*

	No pancreatitis		Pancreatitis	
No. of Stones	M	F	M	F
0–10	64	221	11	4
>10	85	242	14	23

Chi square = 4.1; p = 0.04.

nonpancreatitis group this presentation occurred in 40% of men and 41.3% of women. There was no significant difference in the incidence of acute cholecystitis or jaundice between the two sexes.

Multiple gallstones (>10) were more common in pancreatitic patients ($p < 0.02$). In those with less than 10 stones in their gallbladders, women were less likely than men to get pancreatitis (1.7% *versus* 14.7%) (Table 5). Overall more stones were present in the gallbladders of women (chi square = 9.8, p = 0.002). Men had larger common bile ducts than women; however in those women with a dilated biliary duct system, pancreatitis was more likely to occur (Table 6). Women with pancreatitis had a mean common bile duct diameter on cholangiography of 11.0 mm compared with 8.5 mm in those without this complication. The diameter of the cystic duct was significantly greater in patients with pancreatitis (5.1 ± 1.7 mm *versus* 3.8 ± 1.5 mm) in both men and women (p = 0.0001) (Tables 6 and 7). The range of diameters of this duct, however, was greater in those without pancreatitis than in those with this condition (2 to 18 mm *versus* 1 to 12 mm). As was the case with the common bile duct diameter, males without previous pancreatitis had a larger cystic duct diameter, but both these ducts were bigger in women with pancreatitis than in men.

Pancreatic duct reflux was present on the operative cholangiograms of 62% with pancreatitis, compared with 14% of controls ($p < 0.0001$) and was more common in men (p = 0.01) (Table 8). Men with pancreatic duct reflux had a history of pancreatitis in 42.1%, whereas 19.5% of women with reflux had this complication. The extent of reflux along the pancreatic duct was no greater in men than in women, but was significantly greater in those with a history of pancreatitis in both sexes.

TABLE 6. *Diameter of Common Bile Duct*

	M		F	
Group	No Pancreatitis	Pancreatitis	No Pancreatitis	Pancreatitis
No dilatation	112	15 NS	388	15*
Dilatation	37	10	75	12†

* Chi square = 12.2
† p = 0.0005.

TABLE 7. *Diameter of Cystic Duct (mm)*

Group	Mean	SD	Median	n
M				
No pancreatitis	4.0	2.4	3.2	101
Pancreatitis	4.7	1.5	4.8	16
F				
No pancreatitis	3.6	1.9	3.1	292
Pancreatitis	5.2	2.9	4.3	18

Discussion

That gallstones migrate through the biliary tree and induce pancreatitis by doing so is beyond dispute.[4,5] The factors predisposing to gallstone migration, both through the cystic duct and the sphincter of Oddi, are not well understood.[6] To migrate from the gallbladder into the common bile duct, stones must be of smaller diameter than that of the cystic duct.[6] As they pass through the sphincter of Oddi, impaction at the ampulla probably gives rise to increased pressure and biliary-pancreatic duct reflux.[2,7] This combination of factors may lead to a breach in the tight junctions between cells that form the pancreatic duct mucosal barrier. Small amounts of enterokinase within the bile then may reach the zymogen granules and activate the enzymatic cascade.[8,9] An alternative hypothesis, that of duodenopancreatic duct reflux resulting from ampullary incontinence, consequent on stone migration, also exists.[10,11]

Clearly all stones arising in the gallbladder must begin as minute concretions and slowly increase in size. While very small they presumably are able to pass through the biliary tree with ease, but many grow within the gallbladder and bile ducts before migrating. It is those with diameters of 3 to 7 mm that are most likely to induce pancreatitis by being temporarily arrested in the common channel.[4,5]

For the first time, an additional variable derived from considering the sexes separately has been assessed, which may provide information fundamental to comprehending the pathogenesis of the condition. One basic question that must be asked is whether it is size and shape of the duct system or that of the stone that predisposes to pancreatitis. Clearly the stone is necessary, but we have been able to

TABLE 8. *Pancreatic Duct Reflux Present on Cholangiography*

	No Pancreatitis	Pancreatitis
Reflux	79	25*
M	21	12†
F	58	13†
No reflux	417	20‡

* Chi square = 39.2.
† p = 0.01.
‡ p = 0.0001.

demonstrate no evidence in favor of there being a difference in the stone sizes between the two sexes. Furthermore the number of stones was much greater in women, and the duration for which these stones were present in the biliary tract likewise, but pancreatitis is more common in the man—why? It is likely that anatomic factors are present within the duct system that make the male pancreas more susceptible.

By being larger the duct system of the man has more suitable diameters to permit stones of appropriate size to pass through the cystic duct and become arrested in the ampulla so early in the course of gallstone disease that little alteration in the natural duct size is necessary to allow this migration. Most men had neither dyspepsia, colic, nor cholecystitis before developing pancreatitis. In the woman, however, it is likely that stones need to be present, often for 7 or more years before allowing the critical migration to occur. During this time the duct system slowly increases in size consequent on transient intermittent obstruction, ultimately to permit pancreatitis to develop. The limiting factor is cystic duct size.[6,12] It has been suggested that the cystic duct may only increase in size as a result of increased biliary pressure, transmitted retrogradely by stones becoming arrested close to the ampulla, the narrowest part of the duct system.[6] Despite the increased number of gallbladder stones in the woman, men are more likely to develop pancreatitis, further suggesting that the critical factor in initiating pancreatitis must be duct size and not the size of stones within the gallbladder.

Repeated episodes of stone migration thus would account for the larger duct system present in women with pancreatitis than in those without. In fact by the time pancreatitis occurs, the duct system in the woman is as large or larger than that in the man. Not all women will produce the necessary sequelae of migrating gallstones of gradually increasing size to produce optimum conditions for the development of pancreatitis; hence the man, with a larger duct size *de novo,* has a higher incidence of pancreatitis, as we have shown.

At the critical stone size, the nature of the impaction must be such as to predispose toward bile reflux into the pancreatic duct; bile that is commonly infected in patients with choledecholithiasis.

A rise in pancreatic duct pressure is essential for inducing pancreatitis, for bile alone can commonly enter the pancreatic duct without producing the condition, as evidenced by the frequency of cholangiographic contrast medium entering the pancreatic duct, which is much more common in men than in women and in patients with pancreatitis.[3] This suggests that a second anatomic factor may be present in the man that renders him more susceptible to reflux and may relate to the disposition of the muscle in the sphincter of Oddi at the confluence of the ducts or to the larger diameter of the pancreatic duct in the man with consequently a greatly reduced resistance to back flow.

References

1. Acosta JM, Pellegini CA, Skinner DB. Aetiology and pathogenesis of acute biliary pancreatitis. Surgery 1980; 88:118–125.
2. Armstrong CP, Taylor TV, Jeacock J, Lucas S. The biliary tract in patients with acute gallstone pancreatitis. Br J Surg 1985; 72: 551–555.
3. Taylor TV, Rimmer S. Pancreatic duct reflux in patients with gallstone pancreatitis? Lancet 1980; i:848–851.
4. Acosta JM, Ledesma CL. Gallstone migration as a cause of acute pancreatitis. N Engl J Med 1974; 290:837–840.
5. Kelly TR. Gallstone pancreatitis. Ann Surg 1984; 200:479–485.
6. Taylor TV, Armstrong CP. The migration of gallstones. Br Med J 1987; 294:1320–1322.
7. Armstrong CP, Taylor TV, Jeacock J, Lucas S. The biliary tract in patients with acute gallstone pancreatitis. Br J Surg 1985; 72: 551–555.
8. Creutzfeldt W, Soling HG, Ketterer H. Intrapankreactische Activierung von proteolytischen Fermenten und Trasylolwirkung bei der experimentellen Pankreatitis der Ratte. Klin Wochenschr 1963; 41:1002–1007.
9. Wanke M, Nagel W, Willig F. Formen der experimentellen Pankreatitis patho-anatomisch gesehen. Frankfurter Seitschrift fur Pathologie 1966; 75:207–227.
10. Creutzfeldt W, Schmidt H. Aetiology and pathogenesis of pancreatitis (current concepts). Scand J Gastroenterol 1970; 5:47–62.
11. McCutcheon AD. A fresh approach to the pathogenesis of pancreatitis. Gut 1968; 9:296–310.
12. McMahon MJ, Skefta JR. Physical characteristics of gallstones and the calibre of the cystic duct in patients with acute pancreatitis. Br J Surg 1986; 67:6–9.

The Role of Acetaldehyde in the Pathogenesis of Acute Alcoholic Pancreatitis

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Acetaldehyde (AA), the first product of ethanol metabolism, has been suggested as an important mediator in alcoholic pancreatitis, but experimental evidence has not been convincing. Prior work using the isolated perfused canine pancreas preparation has suggested that toxic oxygen metabolites generated by xanthine oxidase (XO) may mediate the early injury in pancreatitis. Xanthine oxidase is capable of oxidizing AA, and during this oxidation free radicals are released. The hypothesis that acute alcoholic pancreatitis may be initiated by AA in the presence of active XO (converted from xanthine dehydrogenase [XD]) was tested in the authors' experimental preparation by converting XD to XO by a period of ischemia, and infusing AA. Control preparations remained normal throughout the 4-hour perfusion (weight gain, 7 ± 4 g; amylase activity, 1162 ± 202 U/dL). One hour of ischemia, or infusion of AA at 25 mg/hr or at 50 mg/hr without ischemia did not induce changes in the preparation. Acetaldehyde at 250 mg/hr induced minimal edema and weight gain (16 ± 4 g; $p < 0.05$), but not significant hyperamylasemia. Changes also were not observed when 1-hour ischemia was followed by a bolus of ethanol (1.5 g) or sodium acetate (3.0 g), or by infusion of 25 mg/hr of AA. One hour of ischemia followed by infusion of AA at 50 mg/hr or at 250 mg/hr induced edema, hemorrhage, weight gain (22 ± 7 g [$p < 0.05$] and 26 ± 17 g [$p < 0.05$]) and hyperamylasemia (2249 ± 1034 U/dL [$p < 0.05$] and 2602 ± 1412 U/dL [$p < 0.05$]). Moreover infusion of AA at 250 mg/hr after 2 hours of ischemia potentiated the weight gain (62 ± 20 g *versus* 30 ± 14 g [$p < 0.05$]), but not the hyperamylasemia (3404 ± 589 U/dL *versus* 2862 ± 1525 U/dL) as compared with 2 hours of ischemia alone. Pancreatitis induced by 1 hour of ischemia followed by AA at 50 mg/hr could be inhibited by pretreatment with the free radical scavengers superoxide dismutase and catalase and ameliorated with the XO inhibitor allopurinol. The authors conclude that AA, in the presence of active XO, can initiate acute pancreatitis in the isolated canine pancreas preparation and may be important in the initiation of acute alcoholic pancreatitis in man. Toxic oxygen metabolites appear to play an important intermediary role.

THE ASSOCIATION BETWEEN alcohol and acute pancreatitis has been known for over one hundred years,[1] but the mechanisms by which alcohol initiates acute pancreatitis have remained obscure. Several pathogenetic mechanisms have been suggested, including a direct toxic effect of alcohol on the pancreas, and mediation by alcohol induced hyperlipidemia.[2] It has been suggested by some investigators that acetaldehyde, the first stable product of ethanol oxidation, may play a role in the pathogenesis of acute alcoholic pancreatitis,[3-5] although this has not been supported by others.[6] Acetaldehyde is considered one of the major factors in alcohol-induced liver injury,[7] perhaps mediated by free radicals.[7,8] Prior work from this laboratory using the isolated *ex vivo* perfused canine pancreas preparation has suggested that oxygen-derived free radicals generated by xanthine oxidase play an important role in the initiation of experimental pancreatitis.[9,10] Acetaldehyde is an excellent substrate for the enzyme xanthine oxidase, and during its oxidation free radicals are released.[11] We hypothesized that acute alcoholic pancreatitis may be initiated by free radicals from acetaldehyde, generated by xanthine oxidase. To test this hypothesis in our experimental preparation, acetaldehyde was infused after the conversion of xanthine dehydrogenase to xanthine oxidase by a period of ischemia.

Methods

Under general anesthesia (Nembutal, Steris Inc., Chicago, IL) the pancreas with a cuff of duodenum was removed from adult mongrel dogs weighing between 18 and 23 kg. The pancreas was placed in a perfusion circuit in a humified chamber, as previously described.[12] The preparation was perfused through catheters in the splenic and

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superior mesenteric arteries at a constant flow (17 mL/100 g tissue/min). The perfusate was collected in a venous reservoir through a cannula in the portal vein and returned to a Harvey® infant oxygenator (C. R. Bard Inc., Billerica, MA), from where the perfusate was pumped back into the preparation by a roller pump (Watson-Marlow Ltd., England). The temperature of the perfusate was maintained at 37 C with a heat exchanger. The perfusate consisted of 400 mL autologous dog blood, 100 mL Ringer's lactate solution, 0.5 g glucose, 2.5 g human albumin, and 15 mEq sodium bicarbonate. An additional 0.25 g glucose was given after 2 hours of perfusion to maintain the glucose level in the perfusate over 70 mg/dL. An additional 1 to 2 mEq sodium bicarbonate was given when needed to maintain the pH within the physiologic range (7.37 to 7.54).

The perfusion consisted of an initial 30-minute stabilization period, followed by a 4-hour study period. In the ischemic preparations, the perfusion circuit was adjusted to bypass the preparation for either 1 or 2 hours after the stabilization period, but before the 4-hour study period. The preparation was monitored continuously for gross appearance (edema and hemorrhage estimated in the scale none, mild, moderate, or severe), weight, and arterial blood pressure, and measurements were recorded hourly. The perfusate was sampled from the venous line at the beginning of the 4-hour perfusion period (time 0), and hourly thereafter to assay for hematocrit, glucose concentration (automatized blood sugar analyzer, Beckman 2, Beckman, Fullerton, CA) and amylase activity (Amylo-Tube, Pharmacia Diagnostic Inc., Fairfield, NJ). The perfusate was sampled from the arterial line at the same intervals to determine hemoglobin, pH, and blood gases (OSM 2 Hemoximeter and Acid-Base Analyzer, Radiometer, Copenhagen, Denmark). Pancreatic juice was collected with a 16-gauge cannula in the pancreatic duct, and the volume was recorded hourly.

In 10 experiments the perfusate was sampled hourly from both the arterial and the venous lines for assaying acetaldehyde. The sample (0.5 mL) was injected into cooled (0 C), sealed vials containing 0.25 mL 3.0 mol/L perchloric acid. The vials were shaken vigorously, and placed into ice until acetaldehyde was assayed by headspace gas chromatography during the same day. Fresh standards were prepared for every group of experiments.

Student's t test was used to compare the results between groups.

Acetaldehyde—Groups I Through X

Group I.

Controls (6 Preparations). These preparations were perfused for 4 hours after the initial 30-minute stabilization period and monitored as described above. In one preparation the perfusate was sampled for acetaldehyde.

Group II.

Acetaldehyde 25 mg/hr (AA 25) (3 Preparations). These preparations were managed identically to those in group I (controls), except that acetaldehyde (Morton Thiokol Inc., Danvers, MA) was infused at 25 mg/hr into the arterial line over the 4-hour perfusion period starting at time 0. Fresh 5% solution of acetaldehyde in 0.1 mol/L phosphate buffer was prepared at 0 C for every experiment. The infusion syringe was packed in ice to avoid escape of acetaldehyde from the solution (boiling point, 21 C). In three preparations the perfusate was sampled for acetaldehyde.

Group III.

Acetaldehyde 50 mg/hr (AA 50) (8 Preparations). These preparations were managed identically to those in group II, except that acetaldehyde was infused at 50 mg/hr. In three preparations the perfusate was sampled for acetaldehyde.

Group IV.

Acetaldehyde 250 mg/hr (AA 250) (8 Preparations). These preparations were managed identically to those in group II, except that acetaldehyde was infused at 250 mg/hr. Fresh 10% solution of acetaldehyde in 0.1 mol/L phosphate buffer was prepared at 0 C for every experiment. In three preparations the perfusate was sampled for acetaldehyde.

Group V.

One-hour Ischemia (ISCH 1) (5 Preparations). These preparations were managed identically to those in group I (controls), except for a 60-minute period of ischemia between the 30-minute stabilization period and the 4-hour perfusion period.

Group VI.

One-hour Ischemia Followed by Acetaldehyde 25 mg/hr (ISCH 1 + AA 25) (3 Preparations). These preparations were managed identically to those in group V (ISCH 1), except that acetaldehyde was infused over the 4-hour perfusion period at the rate of 25 mg/hr.

Group VII.

One-hour Ischemia Followed by Acetaldehyde 50 mg/hr (ISCH 1 + AA 50) (10 Preparations). These preparations were managed identically to those in group VI (ISCH 1 + AA 25), except that acetaldehyde was infused over the 4-hour perfusion period at the rate of 50 mg/hr.

Group VIII.

One-hour Ischemia Followed by Acetaldehyde 250 mg/hr (ISCH 1 + AA 250) (5 Preparations). These preparations were managed identically to those in group VI (ISCH 1 + AA 25), except that acetaldehyde was infused over the 4-hour perfusion period at the rate of 250 mg/hr.

Group IX.

Two-hour Ischemia (ISCH 2) (5 Preparations). These preparations were managed identically to those in group I (controls), except for a 120-minute period of ischemia between the 30-minute stabilization period and the 4-hour perfusion period.

Group X.

Two-hour Ischemia Followed by Acetaldehyde 250 mg/hr (ISCH 2 + AA 250) (5 Preparations). These preparations were managed identically to those in group IX (ISCH 2), except that acetaldehyde was infused over the 4-hour perfusion period at the rate of 250 mg/hr.

Ethanol and Acetate—Groups XI Through XIV

Group XI.

Ethanol (ETOH) (3 Preparations). These preparations were managed identically to those in group I (controls), except that 1.5 g ethanol as a 10% solution in saline was added to the perfusate at time 0.

Group XII.

One-hour Ischemia Followed by Ethanol (ISCH 1 + ETOH) (4 Preparations). These preparations were managed identically to those in group V (ISCH 1), except that ethanol was added to the perfusate as in group XV (ETOH).

Group XIII.

Acetate (ACE) (3 Preparations). These preparations were managed identically to those in group I (controls), except that 3.0 g sodium acetate (Baker & Adamson, Phillipsburg, NJ; containing three molecules crystallized water in each molecule of sodium acetate) in 10 mL saline was added to the perfusate at time 0.

Group XIV.

One-hour Ischemia Followed by Acetate (ISCH 1 + ACE) (5 Preparations). These preparations were managed identically to those in group V (ISCH 1), except that sodium acetate was added to the perfusate as in group XVII (ACE).

Treatment of Acetaldehyde-induced Injury—Groups XV Through XVII

Group XV.

ISCH 1 + AA 50 + SOD + CAT (5 Preparations). These preparations were managed identically to those in group VII (ISCH 1 + AA 50), except that 50 mg bovine erythrocyte superoxide dismutase (SOD) (Sigma Chemicals Co., St. Louis, MO) and 50 mg bovine liver catalase (CAT) (Calbiochem Corp., La Jolla, CA) were added to the perfusate at the beginning of the stabilization period.

Group XVI.

ISCH 1 + AA 50 + ALLO (5 Preparations). These preparations were managed identically to those in group VII (ISCH 1 + AA 50), except that 100 mg allopurinol (Sigma) were added to the perfusate at the beginning of the stabilization period.

Group XVII.

ISCH 2 + AA 250 + SOD + CAT (4 Preparations). These preparations were managed identically to those in group X (ISCH 2 + AA 250), except that 50 mg SOD and 50 mg CAT were added to the perfusate at the beginning of the stabilization period.

Results

The control preparations (group I) remained normal in gross appearance throughout the 4-hour perfusion period. Weight gain was minimal, and amylase activity in the perfusate remained normal (Tables 1 and 2).

Acetaldehyde infusion at 25 mg/hr (group II) and at 50 mg/hr (group III) did not induce changes in the gross appearance of the pancreas. No significant differences in weight gain or in amylase activity were observed when compared with controls (Tables 1 and 2). When acetaldehyde was infused at 250 mg/hr (group IV), the preparations became mildly edematous, gained weight when compared with the controls, but the amylase activity in the perfusate did not increase significantly (Tables 1 and 2).

One hour of ischemia alone (group V) did not result in changes in gross appearance, weight gain, or amylase activity (Tables 1 and 2). One-hour ischemia followed by acetaldehyde infusion at 25 mg/hr (group VI) also did not induce changes in the preparation (Tables 1 and 2). When 1 hour of ischemia was followed by acetaldehyde at 50 mg/hr (group VII) or at 250 mg/hr (groups VIII), pancreatitis developed with moderate edema and hemorrhage, significant weight gain (Table 1), and hyperamylasemia (Table 2).

Two hours of ischemia alone (group IX) induced pancreatitis with moderate edema, hemorrhage, significant weight gain (Table 1), and an increase in amylase activity (Table 2). Two hours of ischemia followed by acetaldehyde at 250 mg/hr (group X) induced severe pancreatitis with marked edema, severe hemorrhage, high amylase activity (Table 2), and weight gain, which was significantly increased over that induced by a 2-hour period of ischemia alone (Table 1).

Ethanol or acetate alone, or in combination with ischemia (groups XI through XIV), did not induce edema, hemorrhage, weight gain, or significant hyperamylasemia (Tables 3 and 4).

Treatment with SOD and CAT of the pancreatitis induced by 1 hour of ischemia followed by acetaldehyde infusion at 50 mg/hr (group XV) prevented edema, hemorrhage, weight gain, and increase in the amylase activity (Tables 5 and 6). Treatment with SOD and CAT of the more severe pancreatitis induced by 2 hours of ischemia followed by acetaldehyde infusion at 250 mg/hr (group XVII) also significantly ameliorated the weight gain and increase in the amylase activity (Tables 5 and 6). Similarly allopurinol treatment (group XVI) inhibited edema, hemorrhage, and weight gain (Table 5), and ameliorated

TABLE 1. *Weight Gain (g) of* Ex Vivo *Perfused Canine Pancreas Preparations*

Group	n	Time (hr) 0	1	2	3	4
I Control	6	0	2 ± 2	3 ± 3	5 ± 3	7 ± 4
II AA 25	5	0	1 ± 1	2 ± 2	3 ± 5	3 ± 5
III AA 50	8	0	2 ± 3	6 ± 4	6 ± 5	9 ± 6
IV AA 250	8	0	7 ± 6	10 ± 7*	11 ± 4*	16 ± 4*
V ISCH 1	5	0	6 ± 3	8 ± 2	10 ± 4	11 ± 4
VI ISCH 1 + AA 25	3	0	1 ± 1	1 ± 2	3 ± 2	5 ± 3
VII ISCH 1 + AA 50	10	0	5 ± 3	11 ± 3*	17 ± 6*	22 ± 7*
VIII ISCH 1 + AA 250	5	0	6 ± 4	14 ± 11*	20 ± 13*	26 ± 17*
IX ISCH 2	5	0	10 ± 8*	15 ± 10*	20 ± 12*	30 ± 14*
X ISCH 2 + AA 250	5	0	18 ± 6*	34 ± 14*	44 ± 15*	62 ± 20*

Mean ± SD.
Acetaldehyde stimulus with or without preceding ischemia.
* $p < 0.05$ as compared with the control group (I).
Other differences are not significant.
AA, acetaldehyde; ISCH, ischemia.

TABLE 2. *Amylase Activity (U/dL) in the Perfusate of* Ex Vivo *Perfused Canine Pancreas Preparations*

Group	n	Time (hr) 0	1	2	3	4
I Control	6	570 ± 117	705 ± 137	833 ± 111	1031 ± 175	1162 ± 202
II AA 25	5	411 ± 83	498 ± 109	654 ± 239	650 ± 233	831 ± 280
III AA 50	8	599 ± 121	835 ± 356	731 ± 302	1010 ± 224	1365 ± 608
IV AA 250	8	647 ± 194	896 ± 439	1057 ± 542	1368 ± 793	1828 ± 1119
V ISCH 1	5	474 ± 67	911 ± 183	1086 ± 282	1425 ± 181	1445 ± 224
VI ISCH 1 + AA 25	3	624 ± 232	737 ± 351	924 ± 401	838 ± 609	1344 ± 672
VII ISCH 1 + AA 50	10	664 ± 204	1155 ± 515*	1648 ± 798*	1980 ± 1143*	2249 ± 1034*
VIII ISCH 1 + AA 250	5	721 ± 186	1465 ± 814*	2124 ± 1270*	2350 ± 1408*	2602 ± 1412*
IX ISCH 2	5	796 ± 129	1694 ± 227*	2349 ± 970*	3170 ± 2292*	2862 ± 1525*
X ISCH 2 + AA 250	5	929 ± 175	1771 ± 405*	1776 ± 398*	2630 ± 449*	3404 ± 589*

Mean ± SD.
Acetaldehyde stimulus with or without preceding ischemia.
* $p < 0.05$ as compared with the control group (I).
Other differences are not significant.
AA, acetaldehyde; ISCH, ischemia.

TABLE 3. *Weight Gain (g) of* Ex Vivo *Perfused Canine Pancreas Preparations*

Group	n	Time (hr) 0	1	2	3	4
I Control	6	0	2 ± 2	3 ± 3	5 ± 3	7 ± 4
XI ETOH	3	0	5 ± 3	6 ± 3	7 ± 5	9 ± 6
XII ISCH 1 + ETOH	4	0	4 ± 2	7 ± 3	7 ± 5	9 ± 7
XIII ACE	3	0	5 ± 6	5 ± 6	3 ± 1	7 ± 4
XIV ISCH 1 + ACE	5	0	4 ± 4	5 ± 2	8 ± 3	10 ± 4

Mean ± SD.
Ethanol and acetate stimuli with and without preceding ischemia.
Differences are not significant.
ETOH, ethanol; ISCH, ischemia; ACE, acetate.

the increase in amylase activity in the perfusate (Table 6).

In the control preparation, the perfusate contained no detectable acetaldehyde. The acetaldehyde concentration after 4 hours of infusion varied between 320 and 2015 μmol/L in the arterial sample, and between 95 and 1740 μmol/L in the venous sample, depending on the infusion rate (Fig. 1).

In all preparations the arterial pH ranged between 7.36 and 7.53, the PaO_2 between 280 and 550 mm Hg, and the $PaCO_2$ between 28 and 45 mm Hg during the 4-hour perfusion period. Plasma glucose remained over 70 mg/

TABLE 4. *Amylase Activity (U/dL) in the Perfusate of* Ex Vivo *Perfused Canine Pancreas Preparations*

Group	n	Time (hr)				
		0	1	2	3	4
I Control	6	570 ± 117	705 ± 137	833 ± 111	1031 ± 175	1162 ± 202
XI ETOH	3	709 ± 127	858 ± 186	834 ± 134	1038 ± 185	1181 ± 176
XII ISCH 1 + ETOH	4	555 ± 144	760 ± 221	982 ± 404	1248 ± 378	1421 ± 374
XIII ACE	3	541 ± 93	730 ± 258	970 ± 300	1327 ± 563	1611 ± 723
XIV ISCH 1 + ACE	5	775 ± 259	773 ± 387	1002 ± 477	1275 ± 518	1554 ± 577

Mean ± SD.
Ethanol and acetate stimuli with and without preceding ischemia.
Differences are not significant.
ETOH, ethanol; ISCH, ischemia; ACE, acetate.

TABLE 5. *Weight Gain (g) of* Ex Vivo *Perfused Canine Pancreas Preparations*

Group	n	Time (hr)				
		0	1	2	3	4
I Control	6	0	2 ± 2	3 ± 3	5 ± 3	7 ± 4
VII ISCH 1 + AA 50	10	0	5 ± 3	11 ± 3*	17 ± 6*	22 ± 7*
XV ISCH 1 + AA 50 + SOD + CAT	5	0	2 ± 2	3 ± 3†	5 ± 2†	5 ± 3†
XVI ISCH 1 + AA 50 + ALLO	5	0	3 ± 4	4 ± 3†	6 ± 3†	8 ± 4†
X ISCH 2 + AA 250	5	0	18 ± 6*	34 ± 14*	44 ± 15*	62 ± 20*
XVII ISCH 2 + AA 250 + SOD + CAT	4	0	14 ± 11*	20 ± 14*	23 ± 16‡	31 ± 11‡

Mean ± SD.
Pancreatitis was induced by a combination of ischemia and acetaldehyde infusion, and was treated with free radical scavengers SOD and CAT, or xanthine oxidase inhibitor allopurinol.
* $p < 0.05$ as compared with the control group (I).
† $p < 0.05$ as compared with the respective untreated group (VII).
‡ $p < 0.05$ as compared with the respective untreated group (X).
Other differences are not significant.
ISCH, ischemia; AA, acetaldehyde; SOD, superoxide dismutase; CAT, catalase; ALLO, allopurinol.

TABLE 6. *Amylase Activity (U/dL) in the Perfusate of* Ex Vivo *Perfused Canine Pancreas Preparations*

Group	n	Time (hr)				
		0	1	2	3	4
I Control	6	570 ± 117	705 ± 137	833 ± 111	1031 ± 175	1162 ± 202
VII ISCH 1 + AA 50	10	664 ± 204	1155 ± 515*	1648 ± 798*	1980 ± 1143*	2249 ± 1034*
XV ISCH 1 + AA 50 + SOD + CAT	5	571 ± 99	693 ± 165†	812 ± 234†	1017 ± 418†	1087 ± 507†
XVI ISCH 1 + AA 50 + ALLO	5	662 ± 140	853 ± 185	1230 ± 350	1311 ± 307	1480 ± 363
X ISCH 2 + AA 250	5	929 ± 175	1771 ± 405*	1776 ± 398*	2630 ± 449*	3404 ± 589*
XVII ISCH 2 + AA 250 + SOD + CAT	5	837 ± 166	1121 ± 278*‡	1306 ± 225*	1594 ± 222‡	1608 ± 133‡

Mean ± SD.
Pancreatitis was induced by a combination of ischemia and acetaldehyde infusion, and was treated with free radical scavengers SOD and CAT, or xanthine oxidase inhibitor allopurinol.
* $p < 0.05$ as compared with the control group (I).
† $p < 0.05$ as compared with the respective untreated group (VII).
‡ $p < 0.05$ as compared with the respective untreated group (X).
Other differences not significant.
ISCH, ischemia; AA, acetaldehyde; SOD, superoxide dismutase; CAT, catalase; ALLO, allopurinol.

dL throughout the perfusion in all the preparations. In these parameters there were no differences between the various groups. Hemoconcentration was observed in the preparations developing pancreatitis, reaching borderline significance in some of the groups. Arterial pressure declined gradually during the perfusion as peripheral resistance decreased (constant flow) in all the preparations, except in the ISCH 2 + AA 250 group, where the pressure did not decline with the most severe pancreatitis present. The secretory volume of the pancreas was changed significantly from the controls only in the preparations treated with allopurinol, where secretion increased fourfold ($p < 0.02$).

Discussion

Despite decades of investigation, the pathogenesis of acute alcoholic pancreatitis remains obscure. Various hypotheses have been proposed, including biliary-pancreatic

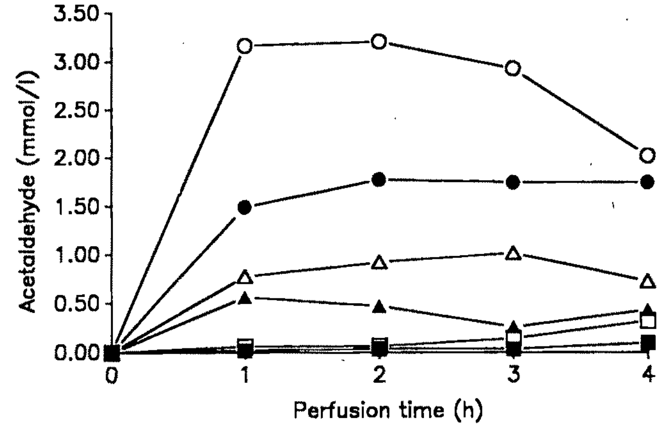

FIG. 1. Acetaldehyde concentration in the perfusate, as sampled in the arterial or venous line, at various rates of acetaldehyde infusion. Mean of three experiments in each group. ○——○, acetaldehyde infusion at 250 mg/hr, arterial sample; ●——●, acetaldehyde infusion at 250 mg/hr, venous sample; △——△, acetaldehyde infusion at 50 mg/hr, arterial sample; ▲——▲, acetaldehyde infusion at 50 mg/hr, venous sample; □——□, acetaldehyde infusion at 25 mg/hr, arterial sample; ■——■, acetaldehyde infusion at 25 mg/hr, venous sample.

reflux, duodenopancreatic reflux, and sphincter of Oddi obstruction with hypersecretion.[2,3] There is little evidence to support these theories, however. The obstruction of pancreatic ductules by protein plugs comes closer to explaining the pathogenesis of chronic pancreatitis than of acute pancreatitis.[3] Previous studies from this institution have provided evidence that hypertriglyceridemia, induced by alcohol abuse, may serve as an intermediary in the initiation of acute pancreatitis.[12] Although this may explain the development of pancreatitis in some alcoholics, it certainly is not the cause in many others.[13] More recently interest has focused on the effects of ethanol on acinar cell metabolism, creating a "toxic metabolic hypothesis."[2] This includes a decrease of trypsin-inhibiting capacity in pancreatic tissue and pancreatic juice, an increase in intracellular ionized calcium, an increase in specific activity of lysosomal enzymes, and an increase in both trypsinogen content and trypsin activity.[2] None of the hypotheses presented thus far is supported by convincing clinical and laboratory data. Experimental acute pancreatitis can not be initiated by giving ethanol to an animal,[14,15] and thus there has been a lack of a suitable experimental model for studying the pathogenesis of acute alcoholic pancreatitis.

In an effort to develop models of acute pancreatitis that simulate the disease as seen in the human, the isolated canine pancreas preparation was adopted.[12,16] Models were developed that simulate acute pancreatitis caused by hyperlipemia,[13] gallstones,[17] and ischemia.[18] An increase in capillary permeability was demonstrated to be the first measurable physiologic response in each of the models studied.[19] Subsequent studies demonstrated this capillary injury to be mediated by toxic oxygen metabolites (free radicals), because the free radical scavengers SOD and CAT inhibited or significantly ameliorated the injury.[9] It was demonstrated that the toxic oxygen metabolites were generated by the enzyme xanthine oxidase, because allopurinol, a specific inhibitor of xanthine oxidase, also inhibited or significantly ameliorated the injury.[10] Because acetaldehyde is known to be an excellent substrate for xanthine oxidase, and during its oxidation toxic oxygen metabolites are generated,[11] we hypothesized that acetaldehyde may initiate acute pancreatitis in many alcoholics in the presence of activated xanthine oxidase.

Xanthine oxidase occurs in tissues primarily as the inactive enzyme xanthine dehydrogenase.[20] Several stimuli are able to induce the conversion of xanthine dehydrogenase to active xanthine oxidase. These include ischemia,[21] hyperthermia,[22] chymotrypsin,[23] trypsin,[24] sulfhydryl oxidase,[25] complement C5,[26] and also acetaldehyde in a dose-dependent manner.[27] In the present experiments, a period of ischemia was used to induce this conversion. Although a 2-hour period of ischemia will by itself initiate acute pancreatitis in the preparation, a 1-hour period of ischemia will not. This has been previously studied and documented in detail.[18] A 1-hour period of ischemia should be enough, however, to induce the conversion of xanthine dehydrogenase to xanthine oxidase.[28]

Infusion of acetaldehyde at 25 mg/hr or at 50 mg/hr, without preceding ischemia, did not induce injury to the isolated pancreas preparation. When acetaldehyde was infused at 50 mg/hr after a 1-hour period of ischemia, however, pancreatitis developed. Pretreatment with SOD and CAT inhibited this injury. In addition acetaldehyde at 250 mg/hr after 2 hours of ischemia potentiated the injury seen after 2 hours of ischemia alone. This injury also could be ameliorated with SOD and CAT. These findings suggest that toxic oxygen metabolites mediated the injury. Moreover because pretreatment with allopurinol also ameliorated the injury response, this suggests that the toxic oxygen metabolites are generated by xanthine oxidase. This supports our hypothesis that acetaldehyde together with xanthine oxidase may initiate acute pancreatitis in our experimental model. The mild edema and minor weight gain after infusion of acetaldehyde at 250 mg/hr without preceding ischemia may be explained by the ability of high acetaldehyde concentrations to induce the conversion of xanthine dehydrogenase to xanthine oxidase.[27]

Acetaldehyde is the first metabolite of ethanol oxidation catalyzed by alcohol dehydrogenase. Ethanol was not injurious to the preparation at concentrations of about 60 mmol/L (calculated), even when administered after 1 hour of ischemia. This suggests that ethanol itself is not metabolized to acetaldehyde in significant amounts in this preparation. This is in agreement with the finding that dog pancreas contains negligible amounts of alcohol dehydrogenase.[29] Interestingly human pancreas has been demonstrated to contain alcohol dehydrogenase.[30]

Acetaldehyde is metabolized to acetate by aldehyde dehydrogenase. Acetate may stimulate the formation of purines, which again may serve as a substrate for xanthine oxidase in the generation of toxic oxygen metabolites.[6] Acetate at concentrations of about 45 mmol/L (calculated), however, was not injurious to the pancreas preparation after 4 hours of perfusion, whether administered with or without preceding ischemia. This suggests that it is acetaldehyde and not its precursor or metabolite that is responsible for the injury.

Previously others have demonstrated that acetaldehyde can be injurious to the pancreas. Intraperitoneal injections of acetaldehyde in the rat increased serum amylase activity and induced structural changes in the acinar cells.[31] Another group demonstrated that when acetaldehyde was infused in the isolated canine pancreas preparation together with secretin stimulation, the pancreas became edematous and hemorrhagic, and secretory volume decreased.[4] In contrast, according to other investigators infusion of acetaldehyde into the splenic artery in the pig did not induce macroscopic or microscopic changes in the pancreas, nor did it affect the amylase activity in the serum.[6] Because acetaldehyde boils at room temperature, and is readily metabolized by several mammalian tissues,[32] including red blood cells,[33] inadequate experimental exposure of the pancreas to acetaldehyde may easily occur. In our studies the presence of acetaldehyde in the perfusate was confirmed by assaying acetaldehyde concentrations in perfusate sampled both in the arterial and venous line. Pancreatitis was observed to develop after 1 hour of ischemia, when acetaldehyde was infused at 50 mg/hr. This infusion rate exposed the pancreas to acetaldehyde at concentrations in the range of 500 μmol/L. In healthy individuals acetaldehyde levels in the peripheral blood are below 10 μmol/L after a moderate test dose of ethanol.[34] The blood acetaldehyde level may exceed 100 μmol/L,[34] however, and may reach even 525 μmol/L,[35] after ethanol abuse. In addition even a low test dose of ethanol may result in blood acetaldehyde concentrations of over 100 μmol/L if an inhibitor for aldehyde dehydrogenase is simultaneously administered.[36] Thus the levels of acetaldehyde achieved in our experimental preparation are what one might expect to find clinically in patients with acute pancreatitis. In humans most of the alcohol dehydrogenase is located in the liver, but some also is found in the pancreas.[30] In addition to blood acetaldehyde originating from liver, some might be produced in the pancreas, possibly resulting in very high local exposure of acinar cells to acetaldehyde. In conclusion these studies suggest that acetaldehyde may play an important role in the pathogenesis of acute alcoholic pancreatitis.

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References

1. Fitz RH. Acute pancreatitis. A consideration of pancreatic hemorrhage, hemorrhagic, suppurative, and gangrenous pancreatitis, and of disseminated fat necrosis. Boston Med Surg J 1889; 120: 181–187, 205–207, 229–235.
2. Singh M, Simsek H. Ethanol and pancreas. Current status. Gastroenterology 1990; 98:1051–1062.
3. Wilson JS, Pirola RC. Pathogenesis of alcoholic pancreatitis. Aust N Z J Med 1983; 13:307–312.
4. Fitzpatrick J, Fitzgerald O, Fitzgerald P, McGeeney KF. The effect of acetaldehyde on the totally isolated canine pancreas. Biol Gastroenterol (Paris) 1975; 8:363.
5. Geokas MC. Ethanol and the pancreas. Med Clin North Am 1984; 68:57–75.
6. Hjorttrop A, Andersen N, Kreutzfeld M, et al. Effect of intra-arterial administration of acetaldehyde on pig pancreas. Dan Med Bull 1983; 30:125–127.
7. Lieber CS. Biochemical and molecular basis of alcohol-induced injury to liver and other tissues. N Engl J Med 1988; 319:1639–1650.
8. Dianzani MU. Role of free radical-mediated reactions in ethanol-induced liver damage. *In* Nordmann R, Ribiere C, Rouach C, eds. Alcohol Toxicity and Free Radical Mechanisms. New York: Pergamon Press, 1988, 35–41.
9. Sanfey H, Bulkley GB, Cameron JL. The role of oxygen derived free radicals in the pathogenesis of acute pancreatitis. Ann Surg 1984; 200:405–413.
10. Sanfey H, Bulkley G, Cameron J. The pathogenesis of acute pancreatitis: the source and role of oxygen derived free radicals in three different experimental models. Ann Surg 1985; 201:633–639.
11. Fridovich I. Oxygen radicals from acetaldehyde. Free Rad Biol Med 1989; 7:557–558.
12. Saharia P, Margolis S, Zuidema GD, Cameron JL. Acute pancreatitis with hyperlipemia: Studies with an isolated perfused canine pancreas. Surgery 1977; 82:60–67.
13. Dickson AP, O'Neill J, Imrie CW. Hyperlipidaemia, alcohol abuse and acute pancreatitis. Br J Surg 1984; 71:685–688.
14. Darle N, Ekholm R, Edlund Y. Ultrastructure of the rat exocrine pancreas after long-term intake of ethanol. Gastroenterology 1970; 58:62–72.
15. Signh M, LaSure MM, Bockman DE. Pancreatic acinar cell function and morphology in rats chronically fed an ethanol diet. Gastroenterology 1982; 82:425–434.
16. Hermon-Taylor J. Chapter 10. *In* Ritchie HD, Hardcastle JD, eds. Isolated Organ Perfusion. Baltimore: University Park Press, 1973.
17. Broe PJ, Cameron JL. Experimental gallstone pancreatitis. Pathogenesis and response to different treatment modalities. Ann Surg 1982; 195:566–573.
18. Broe PJ, Zuidema GD, Cameron JL. The role of ischemia in acute pancreatitis: studies with an isolated perfused canine pancreas. Surgery 1982; 91:377–382.
19. Sanfey H, Cameron JL. Increased capillary permeability: an early lesion in acute pancreatitis. Surgery 1984; 96:485–491.
20. Parks DA, Granger DN. Xanthine oxidase: biochemistry, distribution and physiology. Acta Physiol Scand 1986; suppl 548:87–99.
21. Granger DN. Role of xanthine oxidase and granulocytes in ischemia-reperfusion. Am J Physiol 1988; 255:H1269–H1275.
22. Skibba JL, Powers RH, Stadnicka A, Kalbfleisch JH. The effect of hyperthermia on conversion of rat hepatic xanthine dehydroge-

nase to xanthine oxidase. Biochem Pharmacol 1988; 37:4592–4595.

23. Battelli MG, Lorenzoni E, Stirpe F. Milk xanthine oxidase type D (dehydrogenase) and type O (oxidase). Purification, interconversion and some properties. Biochem J 1973; 131:191–198.
24. Stark K, Seubert P, Lynch G, Baudry M. Proteolytic conversion of xanthine dehydrogenase to xanthine oxidase: evidence against calcium-activated protease (calpain). Biochem Biophys Res Commun 1989; 165:858–864.
25. Clare DA, Blakistone BA, Swaisgood HE, Horton HR. Sulfhydryl oxidase-catalyzed conversion of xanthine dehydrogenase to xanthine oxidase. Arch Biochem Biophys 1981; 211:44–47.
26. Friedl HP, Till GO, Ryan US, Ward PA. Mediator-induced activation of xanthine oxidase in endothelial cells. FASEB J 1989; 3:2512–2518.
27. Sultatos LG. Effects of acute ethanol administration on the hepatic xanthine dehydrogenase/oxidase system in the rat. J Pharmacol Exp Ther 1988; 246:946–949.
28. Parks DA, Granger DN, Bulkley GB, Shah AK. Soybean trypsin inhibitor attenuates ischemic injury to the feline small intestine. Gastroenterology 1985; 89:6–12.
29. Guynn RW, Kuo YJ, Shanbour LL. Acute effect of ethanol on metabolite concentrations of dog pancreas in vivo. Alcoholism 1982; 6:469–474.
30. Buchler R, Pestalozzi D, Hess M, von Wartburg J-P. Immunohistochemical localization of alcohol dehydrogenase in human kidney, endocrine organs and brain. Pharmacol Biochem Behav 1983; 18(suppl 1):55–59.
31. Majumdar APN, Vesenka GD, Dubick MA, et al. Morphological and biochemical changes of the pancreas in rats treated with acetaldehyde. Am J Physiol 1986; 250:G598–G606.
32. Dietrich RA. Tissue and subcellular distribution of mammalian acetaldehyde-oxidizing capacity. Biochem Pharmacol 1966; 15:1911–1922.
33. Beyeler C, Fisch HU, Preisig R. The disulfiram-alcohol reaction: factors determining and potential tests predicting severity. Alcoholism 1985; 9:118–124.
34. Stowell A, Hillbom M, Salaspuro M, Lindros KO. Low acetaldehyde levels in blood, breath and cerebrospinal fluid of intoxicated humans as assayed by improved methods. Adv Exp Med Biol 1980; 132:635–645.
35. Watanabe A, Kobayashi M, Hobara N, et al. A report of unusually high blood ethanol and acetaldehyde in two surviving patients. Alcoholism 1985; 9:14–16.
36. Suokas A, Kupari M, Pettersson J, Lindros K. The nitrefazole-ethanol interaction in man: cardiovascular responses and the accumulation of acetaldehyde and catecholamines. Alcoholism 1985; 9:221–227.

Causes of Renal Allograft Loss

Progress in the 1980s, Challenges for the 1990s

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A variety of refinements in the care of kidney transplant recipients have been instituted over the past decade. The authors studied the overall impact of these refinements on kidney allograft losses at a single institution. To do this they compared the causes and rates of graft loss for primary kidney transplants in the 1970s (January 1, 1970 to December 31, 1979; n = 1012; 657 nondiabetics, 355 diabetics; 617 living donors, 395 cadaver donors) *versus* the 1980s (January 1, 1980 to December 31, 1989; n = 1,384; 756 nondiabetics, 628 diabetics; 740 living donors, 644 cadaver donors). Overall patient survival improved significantly, with rates at 1, 5, and 10 years of 94%, 84%, and 68% for the 1980s, compared with 86%, 69%, and 57% for the 1970s ($p < 0.001$). Actuarial graft survival also improved significantly, with rates at 1, 5, and 10 years of 86%, 71%, and 52% for the 1980s, compared with 73%, 58%, and 43% for the 1970s ($p < 0.001$). This improvement occurred even though there were proportionately more cadaver donors and diabetic recipients in the 1980s. For both decades combined, 24% of the lost grafts were due to chronic rejection, 18% to cardiovascular causes of death with function, 13% to infectious causes of death with function, and 11% to acute rejection. The overall gain in graft survival rates in the 1980s was principally due to fewer cases of acute rejection and fewer infectious deaths. Improvement in graft survival due to the two leading causes—chronic rejection and cardiovascular causes of death—was relatively small, if any. These data indicate that future kidney transplantation research should emphasize prevention of chronic rejection and cardiovascular death.

DURING THE LAST 10 years, a number of modifications in the care of kidney transplant recipients have ensued from our accruing clinical experience and research developments, including alterations in immunosuppressive drugs and protocols, treatment and prevention of infectious diseases, operative indications and techniques, and anesthetic and intensive care management. We decided to examine the net effect of these changes on the rate and causes of graft loss at our institution. We assessed areas where progress has been made, as well as those in which work is now critically needed. To do this we compared outcome and cause of graft loss for primary kidney transplants performed during the 1970s (January 1, 1970 to December 31, 1979) *versus* the 1980s (January 1, 1980 to December 31, 1989).

Methods

From January 1, 1970 to December 31, 1979, 1012 primary kidney transplants (657 nondiabetics, 355 diabetics; 617 living donors, 395 cadaver donors) were performed at the University of Minnesota. We compared this group with the 1384 primary kidney transplants performed here from January 1, 1980 to December 31, 1989 (756 nondiabetics, 628 diabetics; 740 living donors, 644 cadaver donors). The evolution of immunosuppressive protocols and ancillary procedures during this period have been described in detail[1-6] and are depicted in Figure 1. Data on patient characteristics and outcome are stored and continually updated on our computer database.

We tabulated certain patient characteristics potentially relevant to outcome, including recipient age at transplantation, sex, diabetes, primary renal disease, panel reactive antibody (PRA, an index of the level of circulating antibodies against transplantation antigens, maximum and at transplantation), and number of human leukocyte antigen (HLA) loci (A, B, and DR antigens) mismatched with the donor. On October 23, 1990, we calculated actuarial patient and graft survival rates by cause of graft loss and decade of transplantation. Because there were

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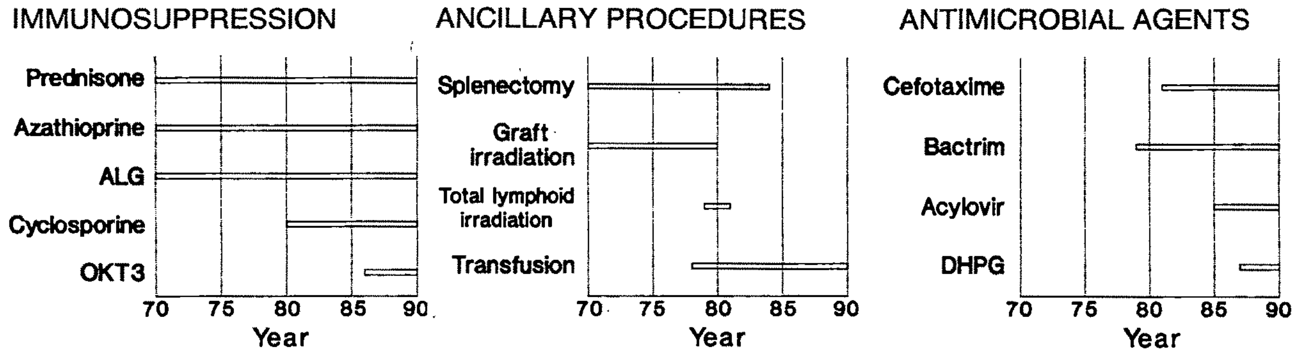

FIG. 1. Evolution of significant changes in the care of kidney recipients over two decades. Prednisone doses gradually have been reduced over the years; azathioprine administration has remained essentially unchanged; ALG was used for all recipients until 1984, and then no longer routinely given to living donor recipients; cyclosporine has been given to nearly all recipients since 1980; OKT3 has been used to treat ALG-resistant rejection and for immune induction of cadaver donor recipients since 1986; splenectomy and graft irradiation were performed routinely in nearly all recipients until 1984 and 1980, respectively; total lymphoid irradiation was practiced for high-risk recipients between 1979 and 1981; transfusion of blood from random donors has been performed preoperatively in nearly all recipients since 1978; cefotaxime, the first third-generation cephalosporine available in the US (1981), is representative of the wide variety of new antibiotics released in the past decade; Bactrim has been administered prophylactically indefinitely to all recipients since 1979; acyclovir has been given as cytomegalovirus prophylaxis to all recipients receiving anti-lymphocyte therapy since 1985; DHPG (ganciclovir) has been used to treat cytomegalovirus disease since 1987.

proportionately more diabetic recipients and cadaver donors in the 1980s, we also analyzed rates by diabetic status and donor source (living or cadaver).

We considered chronic rejection the cause of graft loss in cases showing typical histologic features of chronic rejection on percutaneous renal allograft biopsy (arterial narrowing with concentric fibroblastic thickening, tubular atrophy, interstitial fibrosis, glomerulosclerosis), or a gradual, relentless deterioration of graft function without evidence of obstructive uropathy, renal vascular problems, cyclosporine toxicity, or recurrent disease. Cardiovascular causes of death included myocardial infarction, cerebrovascular accident, congestive heart failure, and cases of sudden death at home presumed to be cardiovascular in nature. Infectious causes of death included generalized sepsis due to bacterial, viral, or fungal organisms, or organ failure due to an infectious agent (*e.g.*, fulminant pneumonia). Acute rejection was the cause of graft loss when graft function deteriorated and failed in the presence of histologic features of ongoing acute rejection (tubulo-interstitial infiltration by lymphocytes, plasma cells, and eosinophils, endothelial swelling with mixed cellular infiltration, interstitial edema).

Patient and graft survival rates and the rate of graft loss due to specific causes were calculated using actuarial techniques. Graft loss was defined as a return to chronic dialysis after transplantation, graft removal, or death of the patient, whichever came first. Patient survival statistics included all deaths, including those that occurred after graft loss. We used the generalized Wilcoxon test of Gehan[7] to compare the two decades' entire survival experience, and the chi square test to compare percentages. Where appropriate we presented numerical data as the mean ± the standard error of the mean (SEM) and used t tests for comparisons. Significant differences occurred when $p < 0.05$.

Results

Patient Characteristics and Overall Survival Rates

Patient demographics are listed in Table 1. The mean age at transplantation in the 1970s (32.9 ± 0.4 years) *versus* the 1980s (33.1 ± 0.4 years) did not differ significantly, nor did sex distribution. There were relatively few diabetic recipients and cadaver donors in the 1970s (35% diabetic, 39% cadaver) compared with the 1980s (45% diabetic, 47% cadaver). The primary renal disease data reflect these differences, with fewer recipients suffering kidney failure because of diabetic nephropathy in the 1970s *versus* the 1980s. The mean PRA in the 1970s (8.8 ± 0.7% maximum, 5.6 ± 0.6% at transplantation) *versus* the 1980s (9.5 ± 0.6% maximum, 4.8 ± 0.4% at transplantation) did not differ significantly.

Actuarial graft and patient survival rates for each decade are shown in Figure 2. Actuarial graft survival rates at 1, 5, and 10 years were 73%, 58%, and 43% for the 1970s *versus* 86%, 71%, and 52% for the 1980s—a highly significant overall difference ($p < 0.001$). Actuarial patient survival rates at 1, 5, and 10 years were 86%, 69%, and 57% for the 1970s *versus* 94%, 84%, and 68% for the 1980s—also highly significant ($p < 0.001$).

Causes of Graft Loss

The percentage distribution of the causes of graft loss within 10 years after transplant for all patients in both decades is shown in Figure 3 and Table 2. The largest proportion (48%) of graft losses was due to death with

TABLE 1. *Patient Demographics*

Variable	Time 1970s	 1980s
Total primary transplants (n)	1012	1384
Age at transplantation (yr)	32.9	33.1
M:F ratio	1:6	1:5
Diabetic:nondiabetic ratio	0:8	1:2
Cadaver:living donor ratio	0:6	0:9
Donor source		
Living donor	617 (60.9%)	740 (53.5%)
HLA-ID relative	146 (14.4%)	180 (13.0%)
Non-HLA-ID relative	471 (46.5%)	548 (39.6%)
Unrelated	0 (0.0%)	12 (0.9%)
Cadaver	395 (39.1%)	644 (46.5%)
Primary renal disease		
Diabetic nephropathy	358 (35.4%)	615 (44.4%)
Glomerulonephritis	255 (25.2%)	201 (14.5%)
Polycystic kidneys	58 (5.7%)	74 (5.3%)
Pyelonephritis	91 (9.0%)	30 (2.2%)
Obstructive uropathy	23 (2.3%)	92 (6.6%)
Hypoplasia	28 (2.8%)	46 (3.3%)
Alport's syndrome	26 (2.6%)	24 (1.7%)
Hypertension	21 (2.1%)	22 (1.6%)
Nephrotic syndrome	7 (0.7%)	13 (0.9%)
Lupus nephritis	18 (1.8%)	16 (1.2%)
Congenital nephrotic syndrome	15 (1.5%)	17 (1.2%)
Interstitial nephritis	4 (0.4%)	20 (1.4%)
IgA nephropathy	1 (0.1%)	22 (1.6%)
Medullary cystic disease	16 (1.6%)	3 (0.2%)
Hemolytic-uremic syndrome	5 (0.5%)	14 (1.0%)
Oxalosis	4 (0.4%)	11 (0.8%)
Other	65 (6.4%)	131 (9.5%)
Unknown	17 (1.7%)	33 (2.4%)
Panel reactive antibody		
Maximum	8.8%	9.5%
At transplantation	5.6%	4.8%

function—including cardiovascular (18%), infectious (13%), and other causes of death (17%)—followed by chronic rejection (24%), acute rejection (11%), and technical complications (5%). The percentage distribution of causes of death in patients with functioning grafts is given in Table 3. The leading causes of graft loss, besides cardiovascular or infectious deaths, were malignancy (7.9% of deaths resulting in graft loss for both decades combined), liver failure (4.6%), and pulmonary embolus (3.8%).

Actuarial curves for rates of graft loss due to the four leading causes (chronic rejection, cardiovascular deaths, acute rejection, and infectious deaths) are shown in Figure 4. The curves depicting graft loss due to chronic rejection for the two decades were statistically different ($p < 0.001$); however, at 10 years, the difference was very small (16% for the 1980s *versus* 18% for the 1970s). Curves were also similar for graft loss due to cardiovascular deaths (Fig. 4B), especially at 10 years (12% for the 1980s *versus* 14% for the 1970s), with no overall statistical difference.

Graft loss due to acute rejection and infectious deaths improved significantly from the 1970s to the 1980s ($p < 0.001$, Fig. 4C and D). At 10 years, graft loss due to acute rejection was the outcome of 9% of transplants in the 1970s, *versus* only 3% in the 1980s. At 10 years, graft loss due to infectious deaths was the fate of 12% of the transplants in the 1970s, *versus* only 4% in the 1980s ($p < 0.001$).

Graft Loss Stratified by Donor Source and Diabetic Status

Because more diabetic and cadaver recipients were transplanted in the 1980s, we repeated our analysis after stratifying the patients by diabetic status and donor source.

Chronic Rejection

Actuarial curves for rates of graft loss due to chronic rejection are shown in Figure 5. Among nondiabetics (both cadaver and living donor recipients), there was no statistically significant difference in rates of graft loss due to chronic rejection in the 1970s *versus* the 1980s (Figs. 5A, B). Among diabetic recipients of cadaver kidneys,

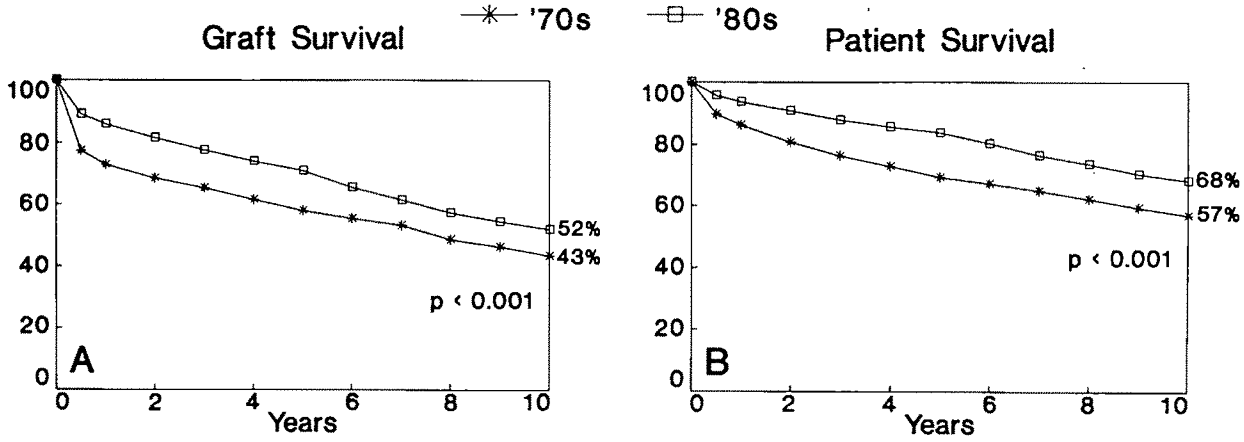

FIG. 2. Actuarial graft and patient survival rates for primary kidney recipients (1970s *vs.* 1980s).

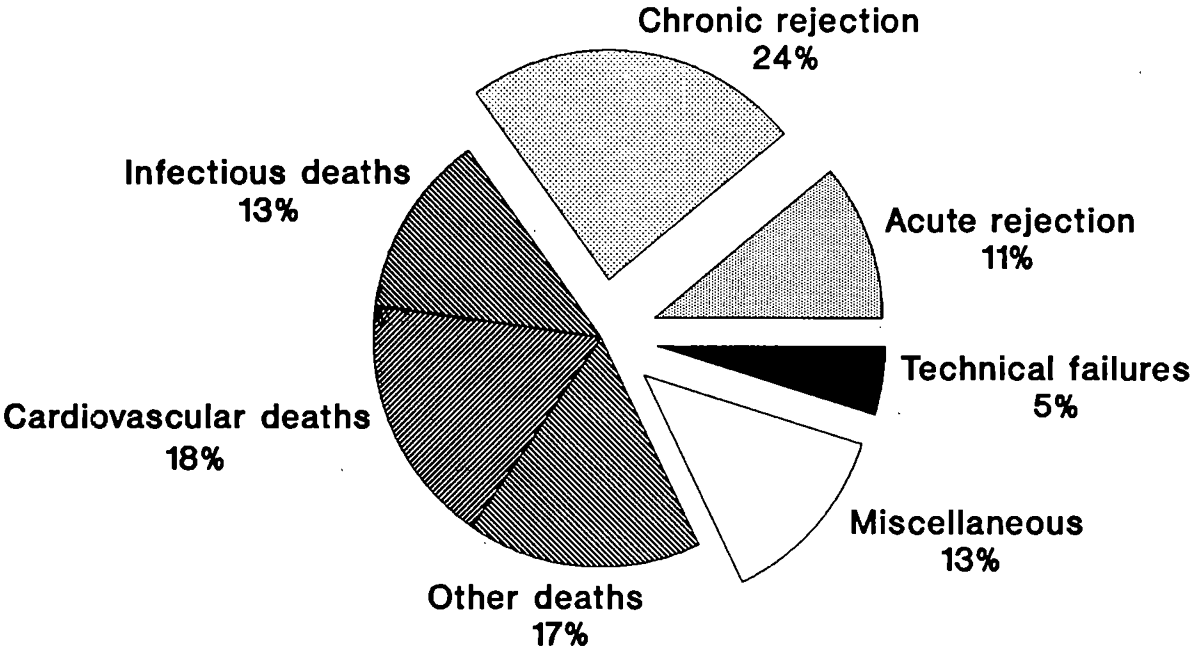

FIG. 3. Causes of graft loss within 10 years after transplant (1970s and 1980s).

there was also no significant change in rates of graft loss due to chronic rejection, but the overall trend appeared to be favorable (Fig. 5C). One subgroup had a statistically significant improvement in rates of graft loss due to chronic rejection—the diabetic recipients of living donor kidneys (Fig. 5D).

Cardiovascular Causes of Death

Actuarial rates of graft loss due to cardiovascular deaths are shown in Figure 6. They were highest among diabetic recipients of cadaver kidneys (Fig. 6C). None of the

TABLE 2. *Causes of Graft Loss Within 10 Years After Transplant*

	Time	
	1970s	1980s
Total grafts lost	680	443
Chronic rejection	157 (23.1%)	111 (25.1%)
Cardiovascular deaths	108 (16.2%)	90 (20.3%)
Infectious deaths	107 (16.2%)	38 (8.7%)
Other deaths	139 (20.4%)	48 (10.8%)
Acute rejection	79 (11.6%)	45 (10.2%)
Technical complications	27 (4.0%)	21 (4.7%)
Miscellaneous		
Recurrent disease	10 (1.4%)	23 (5.1%)
Noncompliance	4 (0.5%)	12 (2.6%)
Vessel thrombosis	3 (0.3%)	10 (2.2%)
Wound infection	9 (1.2%)	3 (0.6%)
Hyperacute rejection	4 (0.5%)	6 (1.4%)
Cortical infarct	4 (0.6%)	6 (1.4%)
Viral sepsis	4 (0.6%)	6 (1.4%)
Acute tubular necrosis	2 (0.3%)	7 (1.6%)
Other infection	7 (1.0%)	1 (0.2%)
Malignancy	2 (0.3%)	3 (0.7%)
De novo disease	0 (0.0%)	3 (0.7%)
Cyclosporine nephrotoxicity	0 (0.0%)	2 (0.5%)
Gastrointestinal bleed	1 (0.1%)	0 (0.0%)
Cortical necrosis	1 (0.1%)	0 (0.0%)
Bacterial sepsis	1 (0.1%)	0 (0.0%)
Fungal sepsis	0 (0.0%)	1 (0.2%)
Urinary extravasation	1 (0.1%)	0 (0.0%)
Unknown	10 (1.4%)	7 (1.6%)

TABLE 3. *Causes of Death Within 10 Years After Transplant*

	Time	
	1970s	1980s
Total grafts lost	344	176
Cardiovascular deaths	108 (31.4%)	90 (51.1%)
Myocardial infarction	52 (15.1%)	35 (19.9%)
Cardiovascular collapse	23 (6.7%)	16 (9.1%)
Cerebrovascular accident	19 (5.5%)	13 (7.4%)
Sudden death	11 (3.2%)	21 (11.9%)
Congestive heart failure	3 (0.9%)	5 (2.8%)
Infectious deaths	107 (31.1%)	38 (21.6%)
Generalized sepsis	57 (16.6%)	21 (11.9%)
Viral sepsis	39 (11.3%)	13 (7.4%)
Pneumonia	11 (3.2%)	4 (2.3%)
Other deaths		
Malignancy	34 (9.9%)	7 (4.0%)
Liver failure	16 (4.7%)	8 (4.5%)
Pulmonary embolus	13 (3.8%)	7 (4.0%)
Diabetic complications	7 (2.0%)	4 (2.3%)
Suicide	6 (1.7%)	3 (1.7%)
Cardiorespiratory arrest	7 (2.0%)	2 (1.1%)
Gastrointestinal hemorrhage	7 (2.0%)	1 (0.6%)
Primary disease	0 (0.0%)	1 (0.6%)
Pulmonary disease	4 (1.2%)	3 (1.7%)
Trauma	6 (1.7%)	1 (0.6%)
Perforated bowel	1 (0.3%)	1 (0.6%)
Bowel obstruction	1 (0.3%)	0 (0.0%)
Hyperkalemia	0 (0.0%)	1 (0.6%)
Pulmonary hemorrhage	1 (0.3%)	0 (0.0%)
Unknown	26 (7.6%)	9 (5.0%)

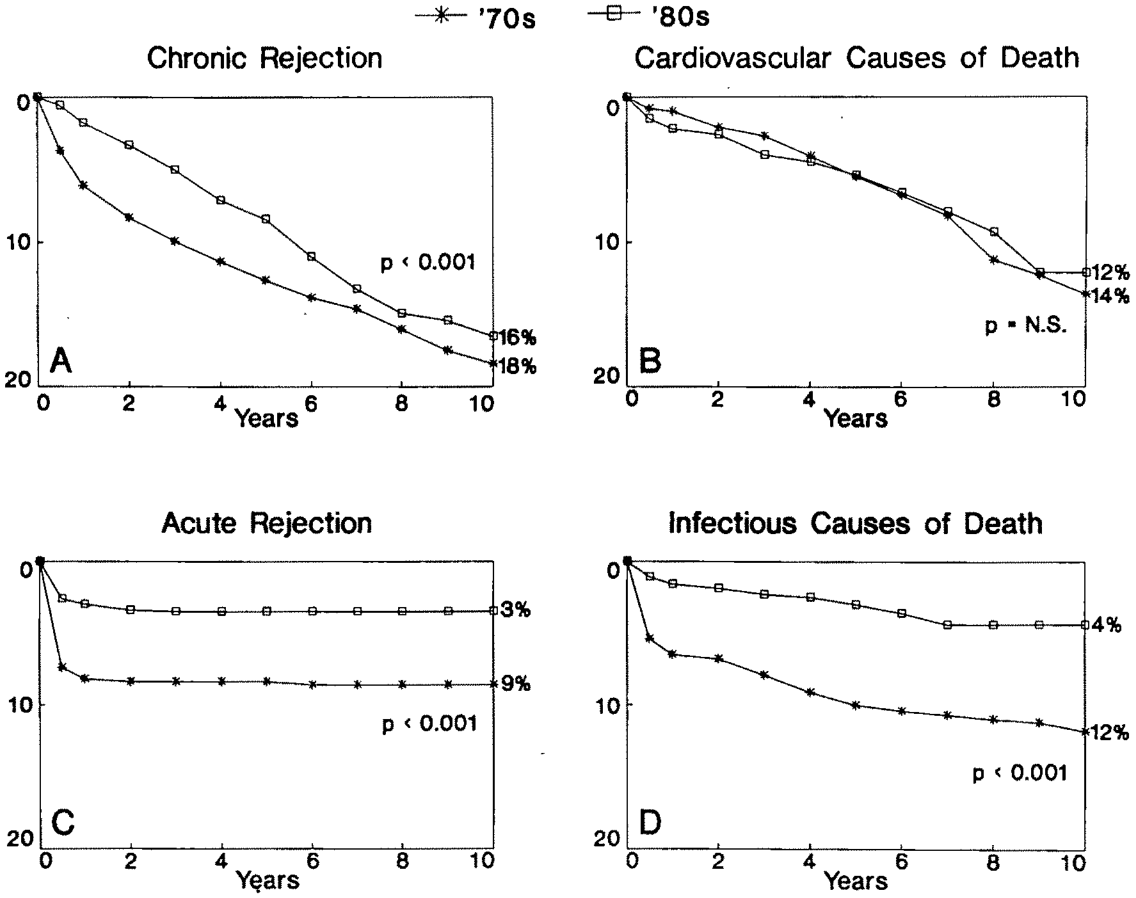

FIG. 4. Actuarial rates of graft loss due to (A) chronic rejection, (B) cardiovascular causes of death, (C) acute rejection, and (D) infectious causes of death for primary kidney recipients (1970s *vs.* 1980s).

subgroups showed statistically significant differences in rates for the 1970s *versus* the 1980s.

Acute Rejection

Actuarial rates of graft loss due to acute rejection are shown in Figure 7. Although diabetic recipients of cadaver kidneys improved most markedly (Fig. 7C), all subgroups showed a statistically significant improvement in the 1980s. Thus the observed overall improvement in graft loss due to acute rejection did not result from differences in the prevalence of diabetic or cadaver donor recipients.

Infectious Death

Actuarial rates of graft loss due to infectious deaths are shown in Figure 8. Again all subgroups showed statistically significant improvement in the 1980s. Thus the observed overall improvement in graft loss due to infectious deaths did not result from differences in the prevalence of diabetic or cadaver donor recipients.

Risk Factors for Chronic Rejection

In an effort to better understand chronic rejection, we stratified all primary kidney recipients (1970s and 1980s combined) by age at transplantation, donor source (living or cadaver donor), number of HLA antigen mismatches, and PRA (maximum and at transplantation), as shown in Table 4. Age at transplantation of patients whose grafts were lost to chronic rejection was significantly lower than that of patients with functioning grafts ($p < 0.001$). At the time of analysis, only 2% of the kidneys donated by HLA-ID siblings were lost to chronic rejection, *versus* 14% from non-ID siblings and 12% from cadaver donors. Similarly the kidneys from HLA-ID donors were more likely to be functioning (74%) *versus* non-ID siblings (52%) and cadaver donors (46%). It therefore appears that HLA-ID kidney recipients are less likely to lose their grafts to chronic rejection.

The influence of HLA matching was less obvious. There was no clear benefit for those patients with fewer mismatches as to the likelihood of having a functioning graft or losing it to chronic rejection. Among patients having no HLA mismatches with their donors, the likelihood of having a functioning graft (65%) or losing it to chronic rejection (3%) was similar to patients with six mismatches (63% and 9%, respectively). There was no difference in the maximum PRA, or that at transplantation, between patients whose grafts were still functioning *versus* those

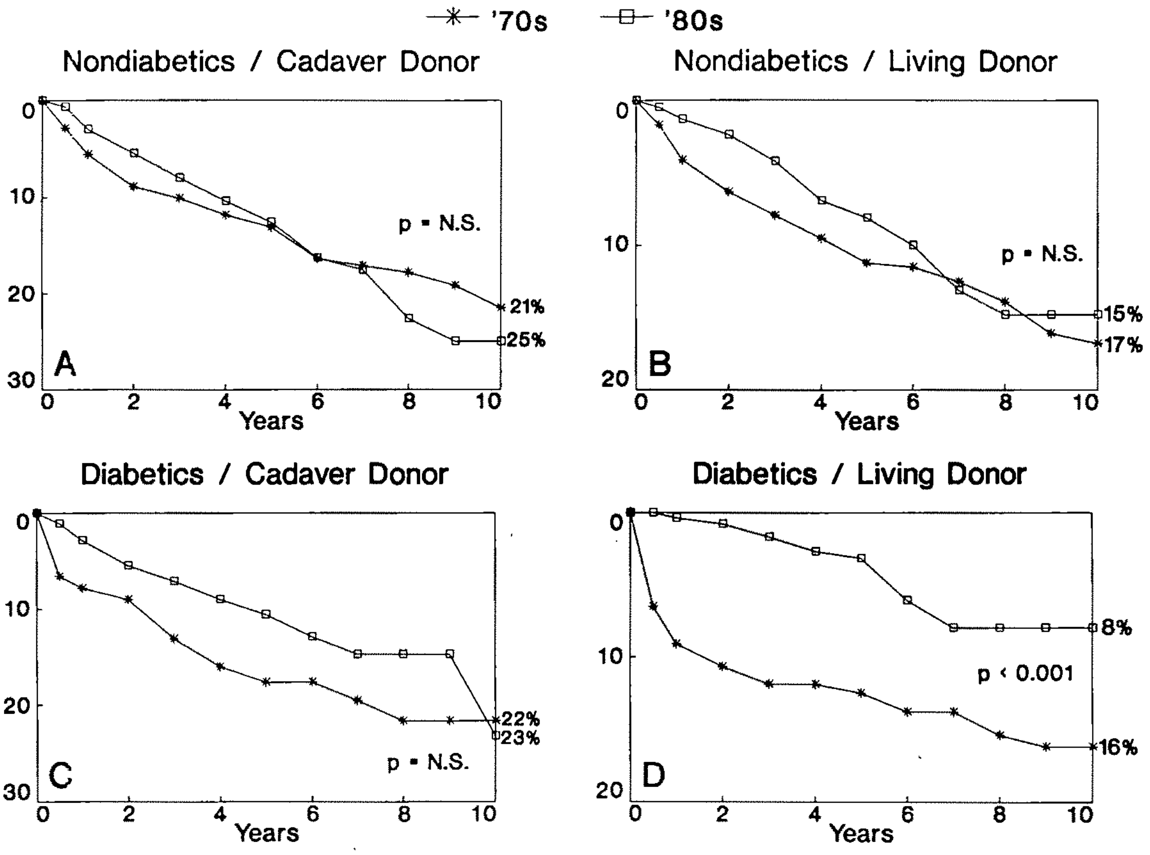

FIG. 5. Actuarial rates of graft loss due to chronic rejection for primary kidney recipients (1970s *vs.* 1980s), stratified by diabetic status and donor source.

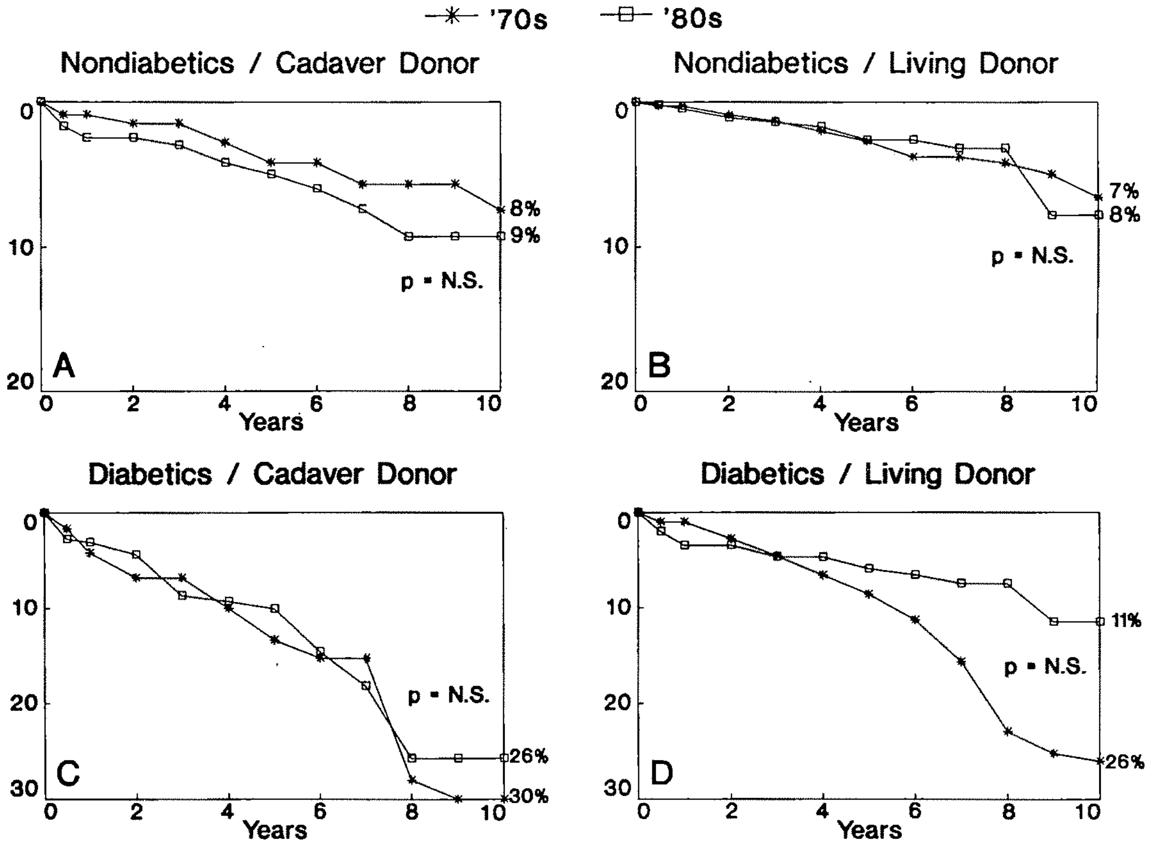

FIG. 6. Actuarial rates of graft loss due to cardiovascular causes of death for primary kidney recipients (1970s *vs.* 1980s), stratified by diabetic status and donor source.

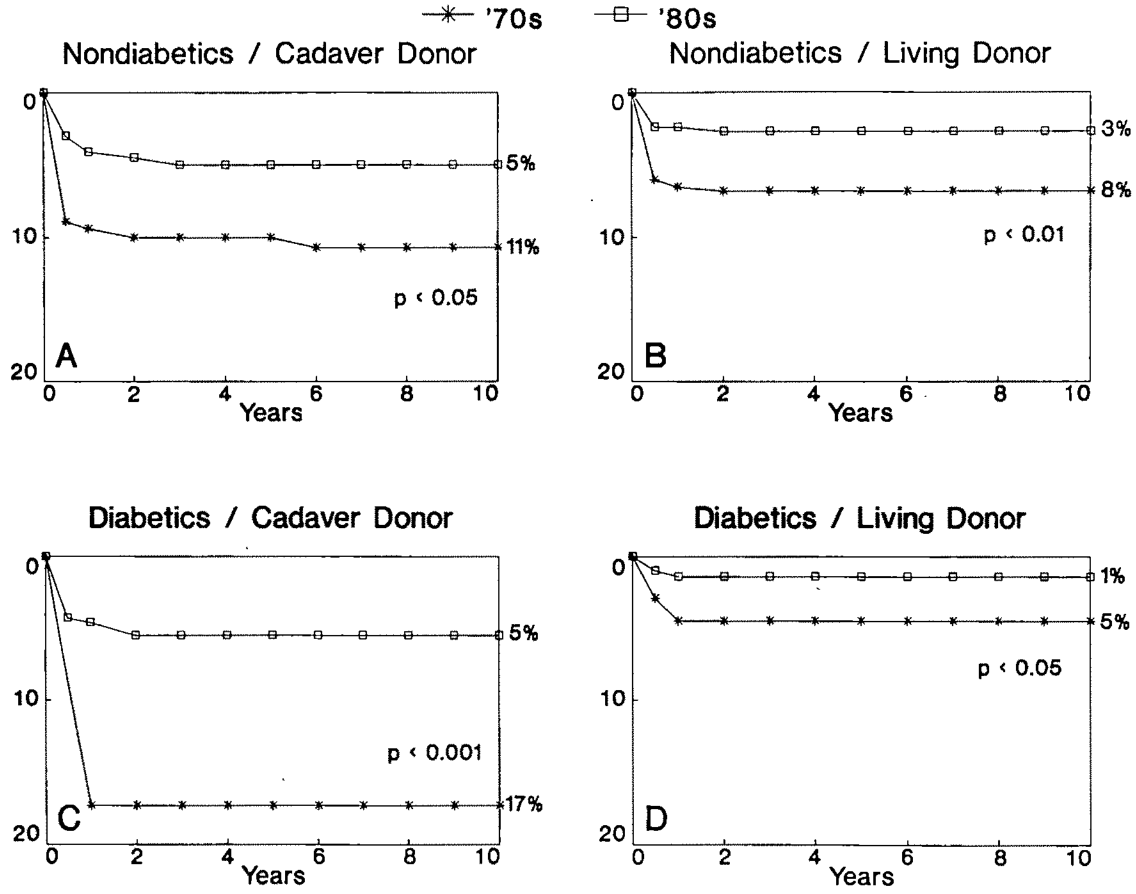

FIG. 7. Actuarial rates of graft loss due to acute rejection for primary kidney recipients (1970s *vs.* 1980s), stratified by diabetic status and donor source.

whose grafts were lost to chronic rejection (p = not significant [NS]). These results agree with other studies showing that the probability of chronic rejection is not a function of the number of HLA antigen mismatches or pretransplant antibody levels.[8] These data raise the possibility of nonimmunologic factors in the development of chronic rejection.

Discussion

Patient and graft survival rates after kidney transplantation have continued to improve over the past decade. Ten years ago, centers with 1-year graft survival rates higher than 66% were said to be "excellent."[9] Now that characterization is reserved for those with graft survival higher than 82%.[10] We designed this study to expose the clinical areas most in need of refinement, to continue the prevailing favorable trend. Our findings reiterate the observation of others that cardiovascular and infectious deaths and chronic rejection are the principal causes of patient and kidney allograft loss.[11-15] But interestingly we found that the advances in overall graft survival rates during the past decade have neither resulted from, nor are reflected in, substantially lower rates of graft loss due to chronic rejection or cardiovascular deaths.

Interval improvements in survival statistics are a consequence of a gradual evolution in clinical practice wherein new, potentially beneficial therapies are implemented and ineffective ones are terminated (Fig. 1). For example we have regularly adjusted our immunosuppressive regimen over the years. Until 1968 only prednisone and azathioprine were used; in 1968 antilymphocyte globulin (ALG) was added.[1] Until 1984 ALG was part of the regimen for all recipients; in 1984 it was discontinued for living donor recipients but retained for cadaver donor recipients. In 1980, cyclosporine was introduced as part of a prospective, randomized clinical trial.[2] When it resulted in fewer rejection episodes, fewer infections, and shorter hospitalization times, cyclosporine joined the "conventional" postoperative immune induction therapy along with prednisone and azathioprine. Other attempts to improve our regimen included progressively reducing the cumulative doses of corticosteroids. Although they have significant side effects, we still consider steroids essential. We are now prospectively evaluating further reduction in dosage to see if it safely results in fewer steroid-induced complications.[16]

We initially treated rejection episodes at our institution with either a high-dose prednisone taper, or three high-

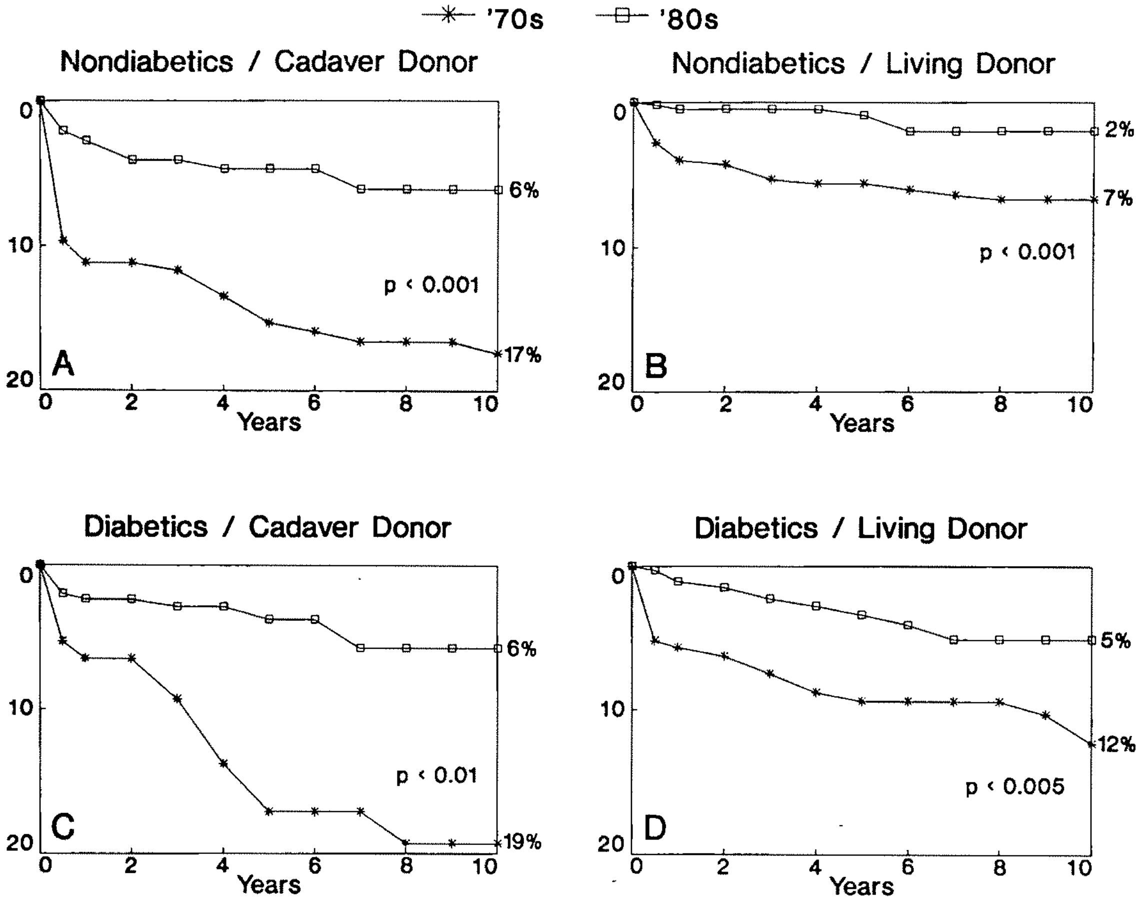

FIG. 8. Actuarial rates of graft loss due to infectious causes of death for primary kidney recipients (1970s *vs.* 1980s), stratified by diabetic status and donor source.

dose bolus injections of methylprednisolone. We currently treat steroid-unresponsive rejection episodes with a 7- to 14-day course of ALG. OKT3, the monoclonal antibody that binds the receptor complex of mature T-lymphocytes, is the most recent addition to our clinical armamentarium for treating rejection episodes.

Several ancillary procedures have been used over the years. We performed routine preoperative splenectomy as an adjunct to immunosuppression from 1970 to 1984. We stopped the practice because a significant morbidity was associated with it,[3] and a prospective study failed to show it was beneficial.[4] We used preoperative fractionated total lymphoid irradiation in high-risk recipients from 1979 to 1981.[6] Although effective it has been supplanted by cyclosporine, which was equally effective in preventing graft loss in these patients.[6] We routinely performed graft irradiation on postoperative days 1, 3, and 5 until the late 1970s. Results did not appear to suffer when it was abandoned, although no controlled evaluation was ever carried out. Finally since 1978 we have done random donor blood transfusions before transplants in nearly all kidney recipients. Although transfusions have been controversial since the advent of cyclosporine,[17-19] they may have contributed to the improved results noted in this study.

Our analysis substantiated the findings of others that chronic rejection is the major cause of graft loss in recipients who outlive their grafts.[11,20] If death with function is excluded,[21] chronic rejection accounts for 45% of all the grafts lost at our institution during the last 20 years. Currently no therapy can arrest or reverse chronic rejection,[22] although adjustments in immunosuppression[23] or diet[24,25] may slow its progression. The prominence of chronic rejection as a cause of graft loss stands in sharp contrast to the small proportion of current textbook chapters and literature devoted to the subject.[26-28] Most available information on chronic rejection was obtained over 10 years ago. There is, in fact, a paucity of new data on the pathogenesis of chronic rejection, especially compared with what has been learned about the mechanisms of acute rejection in the past decade.[29] Because many of the characteristics of chronic rejection are distinct from acute rejection, the two processes need to be studied somewhat independently.The histologic appearance of chronic rejection has been well described.[30,31] It consists of arterial narrowing, glomerulopathy, tubular atrophy, and interstitial fibrosis. Arterial narrowing results from concentric thickening of vessel walls by accumulation of collagen fibrils, myointimal cells, and deposits of finely

TABLE 4. *Characteristics of Primary Kidney Recipients (1970s and 1980s) who Have a Functioning Graft* vs. *Those Whose Graft was Lost to Chronic Rejection*

Variable	Functioning Graft	Graft Lost to Chronic Rejection
Age at transplantation (yr)	32.0	26.2
Donor source		
Living donor		
HLA-ID relative	74% (241/326)	2% (5/326)
Non-HLA-ID relative	52% (530/1019)	14% (143/1019)
Cadaver	46% (480/1039)	12% (120/1039)
No. antigen mismatches		
0	65% (305/468)	3% (14/468)
1	40% (163/407)	16% (65/407)
2	46% (305/669)	14% (95/669)
3	53% (226/427)	12% (49/427)
4	60% (136/227)	11% (24/227)
5	71% (103/145)	10% (15/145)
6	63% (27/43)	9% (4/43)
None typed	20% (2/10)	20% (2/10)
Panel reactive antibody		
Maximum (%)	8.7	10.1
At transplantation (%)	4.9	4.6

granular material.[32] Serial biopsies suggest an immunologically mediated process wherein gamma M immunoglobulin (IgM), complement, platelets, and fibrin deposits are endothelialized and incorporated into the vessel wall.[33,34] As vessels are occluded by this process, ischemia leads to tubular atrophy and interstitial fibrosis. Interleukin-1, released by macrophages infiltrating the graft, may be a pivotal chemical mediator of the fibrous tissue proliferation that characterizes chronic rejection.[29,35]

There is also evidence that nonimmunologic mechanisms are important in allograft failure due to "chronic rejection." These mechanisms are initiated by nephron losses from, for example, acute rejection or prolonged graft ischemia.[8] It is thought that, as nephrons are lost, compensatory hyperfiltration in the residual glomeruli leads to progressive glomerular sclerosis.[36] This latter nonimmunologic aspect of chronic rejection may be exacerbated by a high dietary protein intake.[37] These possible immunologic and nonimmunologic mechanisms on the pathogenesis of chronic rejection are fascinating, but as yet unproven, and in need of critical evaluation.

Cardiovascular deaths constituted the other major cause of graft loss in our patients. The magnitude of this problem is amplified in our series because of the high prevalence of diabetes, a known risk factor for cardiovascular disease. Corticosteroid and cyclosporine administration also may contribute to cardiovascular mortality by unfavorably altering lipid metabolism and aggravating hypertension.[38-40] It would seem that a decline in cardiovascular causes of death among transplant recipients should be sought by reducing risk factors, as in the general population. This would include reduced smoking, better dietary and weight control, exercise programs, and rigorous management of hypertension.

Graft losses due to acute rejection and infectious deaths have declined through the years. This trend was already reported by others in the 1970s,[41,42] and probably has continued into the 1980s because of the favorable effect of superior immunosuppressive regimens and antimicrobial agents.[2,43] Cytomegalovirus (CMV) infection has been a particularly important contributor to mortality rates, morbidity rates, and graft loss, and is also a risk factor for development of acute rejection.[44] The recent availability of antiviral agents like ganciclovir (DHPG, dihydroxypropoxymethylguanine) to treat CMV disease[45] and acyclovir for CMV prophylaxis[46] may have contributed to the reduced infectious mortality rate we noted. Prophylactic trimethoprim-sulfamethoxazole has been administered indefinitely to our recipients since 1979.[47] Although we have not studied its impact in a randomized trial, we believe it has reduced the frequency and morbidity of urinary tract infections,[48] as well as deaths due to sensitive opportunistic organisms such as pneumocystis, nocardia, listeria, and toxoplasma. Finally the recent availability of powerful, less toxic antibiotics such as the third-generation cephalosporins, antipseudomonal penicillins, monolactams, ciprofloxacin, imipenem-cilastatin, and fluconazole, has surely contributed to the observed reduction in infectious deaths.

In summary our study indicates that the net effect of modifications in patient care over the past decade significantly reduced rates of both patient and graft loss. Chronic rejection and cardiovascular deaths are not only the leading causes of graft loss, but also have been least affected by developments in the past decade. Further reducing rates of graft loss is a desirable and attainable goal. The transplant community therefore must focus research efforts on chronic rejection and cardiovascular deaths.

References

1. Najarian JS, Simmons RL, Condie RM, et al. Seven years' experience with antilymphoblast globulin for renal transplantation from cadaver donors. Ann Surg 1976; 184:352–368.
2. Najarian JS, Fryd DS, Strand M, et al. A single institution, randomized, prospective trial of cyclosporine versus azathioprine-antilymphocyte globulin for immunosuppression in renal allograft recipients. Ann Surg 1985; 201:142–157.
3. Matas AJ, Simmons RL, Buselmeier TJ, et al. Lethal complications of bilateral nephrectomy and splenectomy in hemodialyzed patients. Am J Surg 1975; 129:616–620.
4. Sutherland DER, Fryd DS, So SKS, et al. Long-term effect of splenectomy versus no splenectomy in renal transplant patients. Transplantation 1984; 38:619–623.
5. Najarian JS, Ferguson RM, Sutherland DER, et al. Fractionated total lymphoid irradiation as preparative immunosuppression in high risk renal transplantation: clinical and immunological studies. Ann Surg 1982; 196:442–452.
6. Kaufman DB, Kim TH, Slavin S, et al. Long-term clinical studies of high-risk renal retransplant recipients given total lymphoid irradiation. Trans Proc 1989; 21:1798–1799.
7. Gehan E. A generalized Wilcoxon test for comparing arbitrarily singly-censored samples. Biometrika 1965; 52:203–223.
8. Foster MC, Morgan AG, Rowe PA, et al. Characteristics of cadaveric renal allograft recipients developing chronic rejection. Ann R Coll Surg Engl 1990; 72:23–26.

9. Cicciarelli J. Transplant center and kidney graft survival. *In* Terasaki PI, ed. Clinical Kidney Transplants 1985. Los Angeles: UCLA Tissue Typing Laboratory, 1985, 93–110.
10. Benlahrache C, Cecka M, Mickey MR, Cicciarelli J. The center effect. *In* Terasaki PI, ed. Clinical Kidney Transplants 1987. Los Angeles: UCLA Tissue Typing Laboratory, 1987, 325–337.
11. Kirkman RL, Strom TB, Weir MR, Tilney NL. Late mortality and morbidity in recipients of long-term renal allografts. Transplantation 1982; 34(6):347–351.
12. Dlugosz BA, Bretan PN, Novik AC, et al. Causes of death in kidney transplant recipients: 1970 to present. Transplant Proc 1989; 21: 2168–2170.
13. Abele R, Novick AC, Braun WE, et al. Long-term results of renal transplantation in recipients with a functioning graft for 2 years. Transplantation 1982; 34:264–267.
14. Washer GF, Schroter GPJ, Starzl TE, Weil R. Causes of death after kidney transplant. JAMA 1983; 250:49–54.
15. Rosansky SJ, Sugimoto T. An analysis of the United States transplant population and organ survival characteristics: 1977 to 1980. Kidney Int 1982; 22:685–692.
16. Gore SM, Oldham JA. Randomized trials of high- versus low-dose steroids in renal transplantation: does the evidence favor a consensus? Transplantation 1986; 41:319–327.
17. Kahan BD, Kerman RH, Wideman CA, et al. Impact of cyclosporine on renal transplant practice at the University of Texas Medical School at Houston. Am J Kidney Dis 1985; 5:288–295.
18. Opelz G. Effect of HLA matching, blood transfusions, and presensitization in cyclosporine-treated kidney transplant recipients. Transplant Proc 1985; 17:2179–2183.
19. Pfaff WW, Fennell RS, Howard RJ, et al. Planned random donor blood transfusion in preparation for transplantation. Transplantation 1984; 38:701–703.
20. Foster MC, Wenham PW, Rowe PA, et al. The late results of renal transplantation and the importance of chronic rejection as a cause of graft loss. Ann R Coll Surg Engl 1989; 71(1):44–47.
21. Matas AJ, Brayman KL, Gillingham KJ. Presentation of renal transplant survival data: where should "death with a functioning graft" be included? Clinical Transplantation 1990; 4:142–144.
22. Fellstrom B, Larsson E, Tufveson G. Strategies in chronic rejection of transplanted organs: a current view on pathogenesis, diagnosis, and treatment. Transplant Proc 1989; 21(1):1435–1439.
23. Rosenblum ND, Harmon WE, Levey RH. Treatment of chronic renal allograft rejection with cyclosporine and prednisone. Transplantation 1988; 45(1):232–234.
24. Feehally J, Harris KP, Bennett SE, Walls J. Is chronic renal transplant rejection a non-immunological phenomenon? Lancet 1986; 2: 486–488.
25. Sweny P, Wheeler DC, Lui SF, et al. Dietary fish oil supplements preserve renal function in renal transplant recipients with chronic rejection. Nephrol Dial Transplant 1989; 4:1070–1075.
26. G. James Cerilli, ed. Organ Transplantation and Replacement. Philadelphia: JB Lippincott, 1988.
27. Morris PJ, ed. Kidney Transplantation: Principles and practice. 3rd ed. Philadelphia: WB Saunders, 1988.
28. Flye MW, ed. Principles of Organ Transplantation. Philadelphia: WB Saunders, 1989.
29. Braun WE. The immunobiology of different types of renal allograft rejection. *In* Contemporary Issues in Nephrology: Renal Transplantation. New York: Churchill Livingstone, 1989.
30. Porter KA. Renal transplantation. *In* Heptinstall RH, ed. Pathology of the Kidney. Vol. 3. Boston: Little, Brown, 1983.
31. Petersen VP, Olsen TS, Kissmeyer-Nielsen F, et al. Late failure of human renal transplants. An analysis of transplant disease and graft failure among 125 recipients surviving for one to eight years. Medicine 1975; 54:45–71.
32. Porter KA, Rendall JM, Stolinski C, et al. Light and electron microscopic study of biopsies from thirty-three human renal allografts and an isograft 1¾–2½ years after transplantation. Ann NY Acad Sci 1966; 129:615–636.
33. Porter KA, Peart WS, Kenyon JR, et al. Rejection of kidney homografts. Ann NY Acad Sci 1964; 120:472–495.
34. Andres GA, Accinni L, Hsu KC, et al. Human renal transplants: III. Immunopathological studies. Lab Invest 1970; 22:588.
35. Schmidt JA, Mizel SB, Cohen D, Green I. Interleukin 1, a potential regulator of fibroblast proliferation. J Immunol 1982; 128:2177–2182.
36. Hostetter TH, Rennke HG, Brenner BM. Compensatory renal hemodynamic injury: a final common pathway of residual nephron destruction. Am J Kidney Dis 1982; 1:310–314.
37. Brenner BM, Meyer TW, Hostetter TH. Dietary protein intake and the progressive nature of kidney disease. N Engl J Med 1982; 307:652–659.
38. Canadian Transplant Study Group. Examination of parameters influencing the benefit:detriment ratio of cyclosporine in renal transplantation. Am J Kidney Dis 1985; 5:328–332.
39. Canafax DM, Simmons RL, Sutherland DER, et al. Early and late effects of two immunosuppressive drug protocols on recipients of renal allografts: results of the Minnesota randomized trial comparing cyclosporine versus antilymphocyte globulin-azathioprine. Transplant Proc 1986; 18(suppl 1):192–196.
40. Popovtzer MM, Pinnoggera W, Katz FH, et al. Variations in arterial blood pressure after kidney transplantation: relation to renal function, plasma renin activity, and the dose of prednisone. Circulation 1973; 47:1297–1305.
41. Tilney NL, Strom TB, Vineyard GC, Merrill JP. Factors contributing to the declining mortality rate in renal transplantation. N Engl J Med 1978; 299:1321–1325.
42. Salvatierra O, Feduska NJ, Cochrum KC, et al. The impact of 100 renal transplants at one center. Ann Surg 1977; 186:424–435.
43. Strom TM. The improving utility of renal transplantation in the management of endstage renal disease. Am J Med 1982; 73:105–124.
44. Marker SC, Howard RJ, Simmons RL, et al. Cytomegalovirus infection: a quantitative prospective study of three hundred twenty consecutive renal transplants. Surgery 1981; 89:660–671.
45. Collaborative DHPG Treatment Study Group. Treatment of serious cytomegalovirus infections with 9-(1,3-Dihydroxy-2-propoxymethyl)guanine in patients with AIDS and other immunodeficiencies. N Engl J Med 1986; 314:801–805.
46. Balfour HH, Chace BA, Stapleton JT, et al. A randomized, placebo-controlled trial of acyclovir for the prevention of cytomegalovirus disease in recipients of renal allografts. N Engl J Med 1989; 320: 1381–1387.
47. Peters C, Peterson P, Marabella P, et al. Continuous sulfa prophylaxis for urinary tract infection in renal transplant recipients. Am J Surg 1983; 146:589–593.
48. Fox BC, Sollinger HW, Belzer FO, Maki DG. A prospective, randomized, double-blind study of trimethoprim-sulfamethoxazole for prophylaxis of infection in renal transplantation: clinical efficacy, absorption of trimethoprim-sulfamethoxazole, effects on the microflora, and the cost-benefit of prophylaxis. Am J Med 1990; 89:255–274.

Elective and Emergency Hepatic Resection

Determinants of Operative Mortality and Morbidity

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To determine the reasons for improved mortality and morbidity rates after major hepatic resection, five variables were analyzed retrospectively in 300 patients operated on over a 27-year period: (1) the indication for surgery, (2) the surgical approach, (3) the urgency with which surgery was performed, (4) the nature of the surgical procedure, and (5) the experience of the surgeon. The operative mortality rate decreased from 19% between 1962 and 1979 to 9.7% between 1980 and 1988 ($p < 0.05$). The operative mortality rates for patients undergoing resection for benign hepatic neoplasms was 3.4%; for metastatic tumors, 6.3%; for primary hepatic malignancies, 19%; and for trauma, 33%. Fifty-seven percent of operations before 1980 were performed through a thoracoabdominal exposure as compared with 19% after 1980. Overall a thoracoabdominal exposure of the liver was associated with a 20% mortality rate as compared with 8.6% for operations with abdominal exposure of the liver ($p < 0.02$). Elective operations accounted for 65% of hepatic resections before 1980, as compared with 90% after 1980, and were associated with an 8.8% mortality rate as compared with 30.7% for urgent and emergency operations ($p < 0.001$). Segmental and wedge resections were associated with a 5.3% mortality rate as compared with 14.7% for major hepatic resections ($p < 0.05$), but this difference did not affect overall operative mortality rates because there was no change in the proportion of major hepatic resections after 1980. Surgical experience was not a determinant of operative mortality or morbidity rates in elective operations. Although there was no reduction in the complication rate after 1980, there was a reduction in postoperative stay from 26 days before 1980 to 16 days after 1980 ($p < 0.001$). A reduction in the incidence of postoperative sepsis and a change in its management was associated with improved operative mortality rates.

HEPATIC RESECTION benefits patients if the risks of death and complications are outweighed by relief of symptoms and by improved length and quality of survival. This study of hepatic resection assessed the reasons for improved survival rates and reduced morbidity rates over a 27-year period.

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Materials and Methods

Patients

Patients (n = 478) who underwent a liver resection from January 1962 through December 1988 were identified by computer search of all charts in the medical records department and the pathology department for patients undergoing hepatic resection. Three hundred charts were selected for inclusion in this study, representing patients who had had a major hepatic resection, for example, right or left hepatectomy, extended hepatectomy, or segmentectomy. Results of wedge excision of hepatic tumors also were included if surgery was curative. The reasons for exclusion of 178 charts were wedge excision of one of many hepatic metastases (n = 41), liver biopsy examination for benign disease (n = 37), hepatic transplantation (n = 49), minor liver resection *en bloc* with other abdominal malignancy (n = 40), drainage of an abscess or cyst (n = 7), and donor hepatectomy (n = 4).

Demographic, clinical, operative, and radiologic data were collected by review of the hospital chart and of the surgeons' office records. Full data were obtained for 98.4% of patients. An operative death was defined as death attributable to the surgical procedure or to its complications, whether it occurred in the hospital or later. Morbidity was defined as any untoward event during recovery from surgery, as determined by scrutiny of the medical notes and radiologic and biochemical reports.

Classification

Couinaud's classification of hepatic resection was used regardless of the nomenclature used in the operative report.[1,2] Right hepatectomy was removal of segments V,

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VI, VII, and VIII. Extended right hepatectomy removed all of these plus segment IV. Left hepatectomy was removal of segments II, III, and IV. Extended left hepatectomy was resection of segments II, III, and IV with segments V and VIII, or with segments VI and VII. Segmental resection was defined as an anatomic resection of one or more segments including left lateral segmentectomy (excision of segments II and III), and wedge resection as a minor nonanatomic resection.

Operations were classified as *emergency* if the patient's condition necessitated immediate surgery for the control of hemorrhage. If the patient was admitted as an emergency case, but underwent a period of stabilization and investigation before operation, the operation was classified as *urgent*. All other operations were classified as *elective*.

Statistical Analysis

Data were organized into two groups: 1962 through 1979 (18 years, 84 operations) and 1980 through 1988 (9 years, 216 operations). Statistical analysis was performed by the chi square test or by Fisher's exact test for 2 × 2 tables. Student's t test was used to analyze variables in the postoperative stay, and results were expressed as mean ± standard error of the mean (SEM). Statistical significance was stipulated for two-tail p-values < 0.05.

Results

From 1962 to 1988, the number of hepatic resections increased from 29 operations between 1962 and 1970 to 55 operations between 1971 and 1979, and to 216 operations between 1978 and 1988 (Fig. 1).

Deaths

The operative mortality rate in the period 1962 to 1980 was 19%, as compared with 9.7% in the period 1980 to 1988 ($p < 0.05$). Overall the rate of fatal complications was 12.3%. The causes of death were hemorrhage (9), hepatic failure (12), subphrenic sepsis (2), pneumonia (3), septicemia (5), myocardial infarction (1), renal failure (1), and associated trauma (4). Five parameters affecting operative mortality rates were analyzed. These were the indication for surgery, the surgical approach used, the urgency with which surgery was performed, the extent of the surgical procedure, and the experience of the surgeon.

Indication for Resection

The operative mortality rate of patients undergoing resection of benign hepatic neoplasms was 3.4%; for metastatic tumors, 6.3%; for primary hepatic malignancies, 19.1%; and for post-traumatic liver resections, 33.3%. After 1980 the mortality rates were lower for patients with metastatic disease and zero for benign hepatic neoplasms, whereas the mortality rates for primary hepatic malignancy and trauma increased (Table 1). The proportion of liver resections performed for metastatic disease increased from 26% before 1980 to 49% after 1980. Before 1980, 20 liver resections (24%) were performed for trauma as compared with 13 liver resections (6%) after 1980.

Surgical Approach

Four surgical approaches were used for hepatic resection: (1) an abdominal approach (n = 210), (2) a thora-

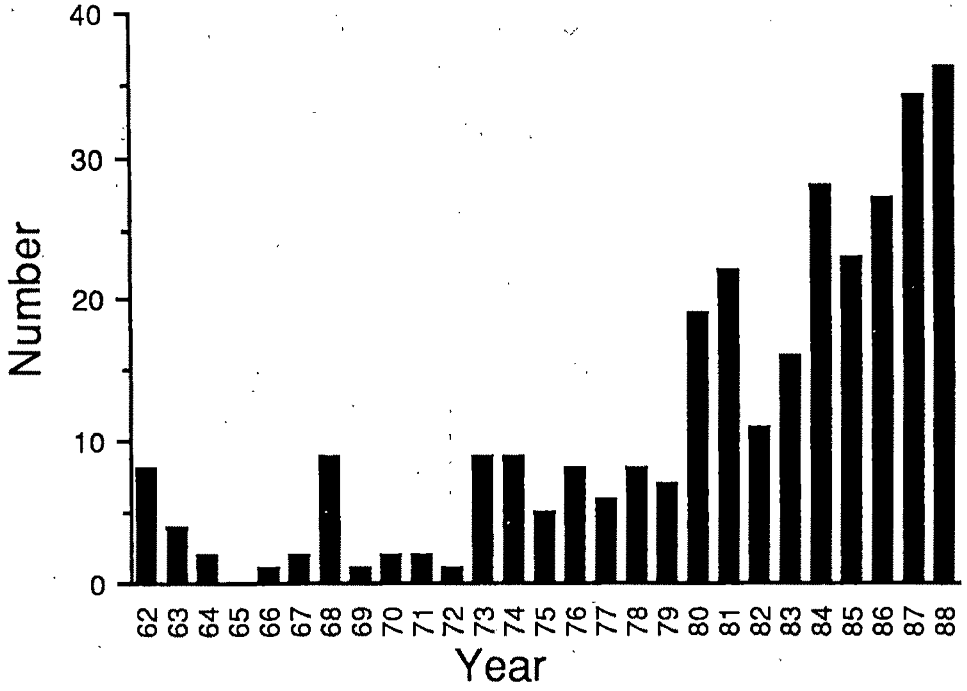

FIG. 1. Number of hepatic resections by year.

TABLE 1. *Operative Death Rate (%OD) for Patients Undergoing Hepatic Resection*

Indication	1962–1988 n	1962–1988 %OD	1962–1979 n	1962–1979 %OD	1980–1988 n	1980–1988 %OD
Primary hepatic malignancy	68	19.1	19	15.8	49	20.4
Hepatocellular carcinoma	42	23.8	14	21.4	28	25.0
Cholangio/gallbladder carcinoma	12	8.3	3	0	9	11.1
Hepatoblastoma	3	0	2	0	1	0
Sarcoma	11	9.1	0	0	11	9.1
Metastases	126	6.3	22	13.6	104	4.8
Benign hepatic tumors	59	3.4	17	11.8	42	0
Hepatic adenoma	12	16.7	2	100	10	0
Focal nodular hyperplasia	15	0	6	0	9	0
Cavernous hemangioma	26	0	5	0	21	0
Benign connective tissue tumor	6	0	4	0	2	0
Hepatic trauma	33	33.3	20	25	13	46.1
Miscellaneous	14	21.4	6	50	8	0

coabdominal approach (n = 65), (3) an abdominal operation extended by a lower sternal split (n = 20), and (4) an abdominal approach extended by a full median sternotomy (n = 5). Thoracoabdominal hepatic resection was associated with a 20.0% mortality rate (n = 90) as compared with 8.6% (n = 210, $p < 0.02$) for abdominal exposure alone. Before 1980 57% of hepatic resections were performed through a thoracoabdominal incision, as compared with 19.4% after 1980 ($p < 0.001$). For both abdominal and thoracoabdominal operations, operative mortality was lower after 1980 (Table 2).

The maximal dimension of tumors resected by a thoracoabdominal approach was 10 cm (median; range, 0.6 to 28 cm), as compared with 6 cm (range, 0.4 to 26 cm) for hepatic resection performed through an abdominal incision alone (NS, Mann-Whitney U test). Solitary tumors were resected in 62% of hepatectomies performed through an abdominal incision as compared with 66% of hepatectomies performed through a thoracoabdominal incision (NS).

Urgency of Operation

Elective operations were associated with a threefold lower mortality rate (8.8%, n = 249) as compared with urgent operations (26.7%, n = 15) or emergency operations (30.6%, n = 36, $p < 0.001$, Table 2). Elective operations accounted for only 65% of hepatic resections before 1980 as compared with 90% of operations after 1980. Urgent or emergency hepatic resection was performed through a thoracoabdominal incision in 49% of patients and through an abdominal incision in 51% (NS).

Nature of Resection

Segmental and wedge resections imposed a mortality rate of only 5.3%, as compared with 14.7% for major resections ($p < 0.05$). No significant differences in mortality rates were associated with different extents of major hepatic resection. The operative mortality rates were lower for all types of hepatic resection except extended right hepatectomy, when comparing the period 1962 to 1979

TABLE 2. *Operative Deaths (%OD), Surgical Approach, and Urgency of Operation*

Variable	1962–1988 n	1962–1988 %OD	1962–1979 n	1962–1979 %OD	1980–1988 n	1980–1988 %OD
Exposure						
Abdominal	210	8.6	36	13.9	174	7.5
Thoracoabdominal						
Lower medial sternotomy	20	15.0	0	0	20	15.0
Thoracoabdominal	65	20.0	46	21.7	19	15.8
Median sternotomy	5	60.0	2	50.0	3	66.7
Urgency of operation						
Elective	249	8.8	55	12.7	194	7.7
Urgent	15	26.7	7	42.9	8	12.5
Emergency	36	30.6	22	27.3	14	35.7
Urgent and emergency	51	29.4	29	31.0	22	27.3
Exposure and urgency						
Elective abdominal	184	6.5	27	3.7	157	7.0
Elective thoracoabdominal	65	15.4	28	21.4	37	10.8
Urgent/emergency abdominal	26	11.5	9	44.4	17	11.8
Urgent/emergency thoracoabdominal	25	36.0	20	25.0	5	80.0

TABLE 3. *Extent of Hepatic Resection and Operative Death Rate (%OD)*

	1962–1988		1962–1979		1980–1988	
Extent of Resection	n	%OD	n	%OD	n	%OD
Major						
Right hepatectomy	102	14.7	28	25.0	74	10.8
Left hepatectomy	52	15.4	18	27.8	34	8.8
Extended right hepatectomy	61	14.7	14	14.3	47	14.9
Extended left hepatectomy	9	11.1	1	100	8	0.0
Total	224	14.7	61	24.6	163	11.0
Minor						
Segmental resection	47	6.4	16	6.2	31	6.4
Wedge resection	29	3.4	7	0.0	22	4.5
Total	76	5.3	23	4.3	53	5.7

with 1980 to 1988 (Table 3). Major resections accounted for 72.6% of hepatic resections before 1980 as compared with 75.5% after 1980 (NS).

Surgical Experience

The range of hepatic resection performed by 54 surgeons was 1-to-131. The operative mortality rate of patients of surgeons who had performed fewer than 10 operations was 16.4%, as compared with 10.0% for more experienced surgeons (NS). For elective operations only, surgeons who had performed fewer than 10 resections had an operative mortality rate of 9.4% as compared with 8.5% for more experienced surgeons. Major resections accounted for 79% of operations performed by surgeons with experience of more than 10 operations, as compared with 67% for less experienced surgeons.

Reoperations

Of 300 patients, 47 (15.7%) underwent 57 reoperations; 15 patients died. The indications for reoperation were postoperative bleeding (18), drainage of subphrenic sepsis (25), exploratory laparotomy (4), operations for wound complications (4), and tracheotomy (8). The reoperation rate was 25% before 1980, as compared with 12.0% after 1980 ($p < 0.005$). In addition, 20 patients (6.7%) underwent 22 percutaneous drainage procedures for intra-abdominal abscesses or collections, and 12 patients (4%) underwent percutaneous drainage of pleural effusions. Before 1980 subphrenic sepsis was treated with reoperation in 15 patients, with a 25% mortality rate. After 1980, subphrenic sepsis was treated by percutaneous drainage in 16 patients and reoperation in 10 patients, with a mortality rate of 2.8%.

Morbidity

Of the 263 patients surviving surgery, 97 (36.9%) patients suffered a total of 209 complications. Local complications comprised subphrenic abscess (20), bile leak (20), postoperative bleeding (9), sterile perihepatic collection (9), enterocutaneous fistula (5), and prolonged ileus (2). Pleural effusion was the commonest pulmonary complication, occurring in 23 patients; other pulmonary complications were pneumonia (7), pneumothorax (4), empyema (3), and respiratory failure requiring tracheotomy (2). Wound infection occurred in 10 patients and wound dehiscence in 2. Metabolic complications occurred in 17 patients; reversible hepatic failure occurred in 14 and renal failure in 3. Of the cardiovascular complications, deep venous thrombosis occurred in two patients and pulmonary emboli in three. Postoperative myocardial infarction and cerebrovascular accident occurred in one patient each. Miscellaneous complications occurred in 27 patients. These included gastrointestinal bleeding (7), septicemia (3), pelvic abscess (2), urinary tract infection (7), and retention of urine (2). Transfusion hepatitis and radial nerve palsy occurred in one patient each.

There was a reduction in the rate of local, pulmonary, and wound complications in the period 1980 to 1988, as compared with 1962 to 1979. Local complications occurred in 29% of patients before 1980, as compared with 20% after 1980. The rate of pulmonary complications was 21.4% before 1980 as compared with 9.7% after 1980. Similarly wound complications occurred in 7.1% of patients before 1980 as compared with 2.8% of patients after 1980.

When analyzed by indication for surgery, there were no significant differences in operative morbidity, comparing 1962 to 1979 with 1980 to 1988. Thoracoabdominal incisions were associated with a morbidity rate of 44.6% as compared with 28.6% for abdominal operations ($p < 0.025$). The rate of pulmonary complications with a thoracic approach was 21.1% as compared with 9.5% for the abdominal approach ($p < 0.01$). Similarly the rate of subphrenic sepsis was 16.5% for thoracic operations as compared with 4.3% for abdominal exposures ($p < 0.02$). There was no significant difference in the morbidity rate for elective operations (31.7%) as compared with the rate for urgent or emergency operations (35.3%).

Extended hepatectomy was associated with a morbidity rate of 45.7% ($p < 0.01$), as compared with 28.3% for all other operations ($p < 0.01$). Segmental or wedge resections were associated with the lowest morbidity rate (15.8%, $p < 0.001$). There were no differences in operative morbidity when analyzed by experience of the surgeon.

Postoperative Stay

The mean (±SEM) postoperative stay was 18 ± 1.0 days. Before 1980 the postoperative stay was 26 ± 2.5 days, as compared with 16 ± 0.9 days after this date ($p < 0.001$). The postoperative stay over the final 2 years of the study fell to 13 ± 1.0 days. The postoperative stay for elective operations was 17 ± 0.8 days, as compared with 29 ± 4.0 days for urgent or emergency operations ($p < 0.01$). After major hepatic resections, the postoperative stay was 20 ± 1.3 days, compared with 14 ± 1.1 days for segmental or wedge excisions ($p < 0.001$).

Discussion

At our hospital five trends in hepatic surgery were associated with reduced operative mortality rates over a 27-year period: an increase in the frequency of operations, a change in the indications for surgery with an increase in the proportion of operations for lower-risk indications, a trend away from resection of the liver through a thoracoabdominal incision, an increase in elective surgery, and a change in the incidence and management of postoperative sepsis. The nature or extent of the operative procedure and the experience of the surgeon, however, were not associated with the improvement in operative mortality seen in recent years.

Hepatic resection is now performed more frequently because operative mortality rates have fallen in the last 30 years. In the 1960s operative mortality rates of 36%,[3] 35%,[4] and 29%[5] were reported. The poor long-term survival and high operative mortality rates of patients undergoing hepatic resection for metastases led to the belief that surgery was indicated for the palliation of symptoms only.[5] One study in 1963, however, demonstrated that resection of hepatic metastases in suitably selected cases was attended by an operative mortality of only 4% and a 5-year survival rate of 30%,[6] and subsequent studies have reported a reduction in operative mortality rate in recent years for hepatic resection.[7-11]

The increased frequency of hepatic resection and improvement in operative mortality was associated with a relative change in the indications for operation. For patients undergoing liver resection of metastases from colorectal cancers, operative mortality rates under 10%[12-23] and between 10% and 15% have been reported.[24-26] The 5-year survival of patients undergoing resection of hepatic metastases from colorectal cancer is 25%.[27] Metastases to a resectable part of the liver from a colorectal primary cancer therefore have become established as an indication for liver resection; this event is reflected in a rise in both the total number and in the proportion of hepatic resections performed for this indication. Because hepatic resection for metastatic disease is attended by a lower operative mortality rate than is hepatic resection for trauma and primary malignant hepatic neoplasms, the consequence of the increase in hepatic resections for colorectal cancer metastases is a reduction in overall operative mortality rate for hepatic resection.

Hepatic resection for benign hepatic neoplasms is attended by operative mortality rates under 6%.[3,28-31] In contrast hepatic resection for primary hepatic malignancy and trauma is associated with greater operative risk. For hepatocellular carcinoma and other primary malignant neoplasms, an early review reported an operative mortality rate of 20%.[32] Operative mortality rates between 20% and 30%[8,33,34] and 10% to 20%[35-42] are more frequently encountered than operative mortality rates under 10%[43-50] for resection of primary malignant hepatic neoplasms. The reduction in overall operative mortality rates seen after 1980, however, was not related to a change in the proportion of patients undergoing resection for benign or primary malignant hepatic neoplasms, because the proportion of resections performed for these indications remained constant.

Although operative mortality rates for patients sustaining injuries to the liver is reported between 5% and 13%,[11,51-53] for the small number of patients requiring hepatic resection for such injuries, the operative mortality rates are 36%,[54] 47.6%,[55] and 83%.[56] We report an overall operative mortality rate of 33% for patients undergoing hepatic resection for hepatic trauma: four of the eleven patients died from associated injuries and, when these deaths are excluded, the operative mortality rate was 21%. The mortality rate of hepatic trauma has been reported to parallel the number of associated injuries.[51] Although we report an increase in the operative mortality rate of the small number of patients undergoing hepatic resection for hepatic trauma after 1980, the reduction in the relative proportion of patients undergoing hepatic resection for trauma reduced the overall operative mortality rate for hepatic resection.

Advances in surgical technique, as shown by a trend away from the use of a thoracoabdominal incision for liver resection, are also a major influence on operative mortality rates. A thoracoabdominal approach to liver resection was associated with a 4% operative mortality rate in 1963.[6] Since this time, however, the use of a thoracoabdominal approach has been associated with a twofold increase in operative blood loss and blood transfusion requirements[57] and an increase in mortality and morbidity rates.[13,57,58] A thoracic extension is now almost never required for hepatic resection.[59]

Although we report a reduction in operative mortality rates for both abdominal and thoracoabdominal operations after 1980, the operative mortality rate for thoracoabdominal operations was twice that for abdominal operations even after 1980. The use of a thoracic extension to the incision may indicate a more complex or difficult operation. There were no differences in the size or number of tumors resected by the abdominal approach as compared with the thoracoabdominal approach, however; surgical preference appeared to be the determinant of the choice of incision. The trend toward abdominal exposure of the liver therefore was a major influence on overall operative mortality rates.

Emergency operations are attended by higher operative mortality rates than are elective operations.[54,60] Such operations are often performed in patients whose general condition is poor and who require surgery to save life or palliate symptoms. The increasing frequency with which hepatic resection is performed was associated with an increase in the proportion of elective operations. The threefold lower mortality rate associated with elective hepatic resection therefore was a major factor in the overall reduction in operative mortality rates.

We report, as have others,[40,61–65] that segmental or wedge resection was associated with a mortality rate half that of major hepatic resection. Minor resections of the liver for metastatic cancer are not associated with worse long-term survival.[66] Because there was no trend toward segmental and wedge resection of the liver over the 27-year period, however, the extent of surgical procedure did not influence the operative mortality rate.

Greater surgical experience may be expected to be associated with lower operative mortality rates after hepatic resection. More experienced surgeons, however, may undertake surgery in higher-risk patients or in patients whose lesions are difficult to excise. Lower operative mortality rates were found in the patients of surgeons who had performed more than 10 hepatic resections. Nonetheless this difference was less evident when the data were analyzed for elective operations only and suggests that a greater proportion of emergency resections were performed by less experienced surgeons. A greater proportion of resections performed by surgeons with experience of more than 10 operations were major resections, and the higher operative mortality rates of such resections may account for the small differences in operative mortality rates between the more experienced and the less experienced surgeons. Therefore for elective hepatic resection, the number of hepatic resections performed by a surgeon is not a guide to operative mortality rates.

Morbidity rates after hepatic resection may be determined by the complication rate, the reoperation rate, and the length of postoperative stay. Complication rates of between 12.5%[13] and 62%[23] have been reported after hepatic resection and are higher after major hepatic resection.[14,63,67] Although we report no change in complication rates over the 27-year period, the reoperation rate, the length of postoperative stay, and the rates of pulmonary complications and subphrenic sepsis have fallen. Because both pulmonary complications and subphrenic sepsis rates are greater with thoracoabdominal exposure of the liver, the reduction in these complications was due to the trend away from this approach to liver resection.

A change in the management of complications after hepatic resection also has resulted in reduced operative mortality rates. Open drainage of subphrenic sepsis was associated with a 25% mortality rate before 1980. After 1980, a combination of percutaneous drainage techniques and open operation was associated with much lower operative mortality rates. These data are consistent with a previous report showing a 37% mortality rate associated with reoperation and 14% mortality rate for sepsis treated initially with percutaneous drainage after hepatic resection.[54]

Conclusion

Adson and Weiland[49] stated that "the risk of routine or standardized major hepatic resection is less than 3 percent. However the risk involved in trying too hard or in resecting inadvisedly is 50 percent or more." We report data consistent with this statement. The recent reduction in operative mortality rate is associated with an increase in the frequency of hepatic resection, an increase in the proportion of elective operations for lower-risk indications, and a change in the surgical approach away from thoracoabdominal incisions. Although complication rates have changed little, a reduction in the reoperation rate and postoperative stay demonstrate the decrease in morbidity rates in recent years from hepatic resection.

References

1. Couinaud C. Le foie: études anatomiques et chirurgicales. Paris: Masson & Cie, 1957, pp 283–289.
2. Malt RA. Surgery for hepatic neoplasms. N Engl J Med 1985; 313: 1591–1596.
3. Gans H, Koh S-K, Aust JB. Hepatic resection: results in 39 patients operated upon during the 11-year period from 1952 to 1963. Arch Surg 1966; 93:523–530.
4. Schweizer O, Howland WS. Anesthetic management during total hepatic lobectomy. Surg Gynecol Obstet 1960; 110:61–65.
5. Brunschwig A. Hepatic lobectomy for metastatic cancer. Cancer 1963; 16:277–282.
6. Woodington GF, Waugh JM. Results of resection of metastatic tumors of the liver. Am J Surg 1963; 105:24–29.
7. Fortner JG, Maclean BJ, Kim DK, et al. The seventies evolution in liver surgery for cancer. Cancer 1981; 47:2162–2166.
8. Lee NW, Wong J, Ong GB. The surgical management of primary carcinoma of the liver. World J Surg 1982; 6:66–75.
9. Bengmark S, Hafström L, Jeppsson B, Sundqvist K. Primary carcinoma of the liver: improvement in sight? World J Surg 1982; 6:54–60.
10. Boerma EJ. Research into the results of resection of hilar bile duct cancer. Surgery 1990; 108:572–580.
11. Defore WW Jr, Mattox KL, Jordan GL Jr, Beall AC Jr. Management of 1,590 consecutive cases of liver trauma. Arch Surg 1976; 111: 493–497.

12. Bradpiece HA, Benjamin IS, Halevy A, Blumgart LH. Major hepatic resection for colorectal liver metastases. Br J Surg 1987; 74:324–326.
13. Nordlinger B, Parc R, Delva E, et al. Hepatic resection for colorectal liver metastases. Ann Surg 1987; 205:256–263.
14. Gennari L, Doci R, Bozzetti F, Bignami P. Surgical treatment of hepatic metastases from colorectal cancer. Ann Surg 1986; 203: 49–54.
15. Ekberg H, Tranberg K-G, Andersson R, et al. Determinants of survival in liver resection for colorectal secondaries. Br J Surg 1986; 73:727–731.
16. Bengmark S, Hafström L, Jeppsson B, et al. Metastatic disease in the liver from colorectal cancer: an appraisal of liver surgery. World J Surg 1982; 6:61–65.
17. Holm A, Bradley E, Aldrete JS. Hepatic resection of metastasis from colorectal carcinoma. Ann Surg 1989; 209:428–434.
18. Fortner JG, Silva JS, Golbey RB, et al. Multivariate analysis of a personal series of 247 consecutive patients with liver metastases from colorectal cancer: 1. Treatment by hepatic resection. Ann Surg 1984; 199:306–316.
19. Wilson SM, Adson MA. Surgical treatment of hepatic metastases from colorectal cancers. Arch Surg 1976; 111:330–334.
20. Adson MA, van Heerden JA. Major hepatic resections for metastatic colorectal cancer. Ann Surg 1980; 191:576–583.
21. Logan S, Meier SJ, Ramming KP, et al. Hepatic resection of metastatic colorectal carcinoma. Arch Surg 1982; 117:25–28.
22. Coppa GF, Eng K, Ranson JHC, et al. Hepatic resection for metastatic colon and rectal cancer. Ann Surg 1985; 202:203–208.
23. Vetto JT, Hughes KS, Rosenstein R, Sugarbaker PH. Morbidity and mortality of hepatic resection for metastatic colorectal carcinoma. Dis Colon Rectum 1990; 33:408–413.
24. Rajpal S, Dasmahapatra KS, Ledesma EJ, Mittelman A. Extensive resections of isolated metastasis from carcinoma of the colon and rectum. Surg Gynecol Obstet 1982; 155:813–816.
25. Petrelli NJ, Nambisan RN, Herrera L, Mittleman A. Hepatic resection for isolated metastasis from colorectal carcinoma. Am J Surg 1985; 149:205–209.
26. Fegiz G, Bezzi M, de Angelis R, et al. Surgical treatment of liver metastases from colorectal cancer. Ital J Surg Sci 1985; 15:259–265.
27. Hughes K, Scheele J, Sugarbaker PH. Surgery for colorectal cancer metastatic to the liver. Surg Clin North Am 1989; 69:339–359.
28. Sesto ME, Vogt DP, Hermann RE. Hepatic resection in 128 patients: a 24-year experience. Surgery 1987; 102:846–851.
29. Iwatsuki S, Todo S, Starzl TE. Excisional therapy for benign hepatic lesions. Surg Gynecol Obstet 1990; 171:240–246.
30. Longmire WP Jr, Trout HH III, Greenfield J, Tompkins RK. Elective hepatic surgery. Ann Surg 1974; 179:712–721.
31. Tomás-de la Vega JE, Donahue EJ, Doolas A, et al. A ten year experience with hepatic resection. Surg Gynecol Obstet 1984; 159:223–228.
32. Foster JH. Survival after liver resection for cancer. Cancer 1970; 26: 493–502.
33. Harrison NW, Dhru D, Primack A, et al. The surgical management of primary hepatocellular carcinoma in Uganda. Br J Surg 1973; 60:565–569.
34. Inouye AA, Whelan TJ. Primary liver cancer: a review of 205 cases in Hawaii. Am J Surg 1979; 138:53–61.
35. Lai EC-S, Ng IO-L, Ng MM-T, et al. Long-term results of resection for large hepatocellular carcinoma: a multivariate analysis of clinicopathological features. Hepatology 1990; 11:815–818.
36. Gozzetti G, Mazziotti A, Cavallari A, et al. Clinical experience with hepatic resections for hepatocellular carcinoma in patients with cirrhosis. Surg Gynecol Obstet 1988; 166:503–510.
37. Nagasue N, Yukaya H, Chang Y-C, et al. Assessment of pattern and treatment of intrahepatic recurrence after resection of hepatocellular carcinoma. Surg Gynecol Obstet 1990; 171:217–222.
38. Kanematsu T, Takenaka K, Matsumata T, et al. Limited hepatic resection effective for selected cirrhotic patients with primary liver cancer. Ann Surg 1984; 199:51–56.
39. Nagao T, Goto S, Kawano N, et al. Hepatic resection for hepatocellular carcinoma. Ann Surg 1987; 205:33–40.
40. Okamoto E, Tanaka N, Yamanaka N, Toyosaka A. Results of surgical treatments of primary hepatocellular carcinoma: some aspects to improve long-term survival. World J Surg 1984; 8:360–366.
41. Bismuth H, Houssin D, Ornowski J, Meriggi F. Liver resections in cirrhotic patients: a western experience. World J Surg 1986; 10: 311–317.
42. Sørensen TIA, Aronsen K-F, Aune S, et al. Results of hepatic lobectomy for primary epithelial cancer in 31 adults. Am J Surg 1979; 138:407–410.
43. The Liver Cancer Study Group of Japan. Primary liver cancer in Japan: clinicopathologic features and results of surgical treatment. Ann Surg 1990; 211:277–287.
44. Tsuzuki T, Sugioka A, Ueda M, et al. Hepatic resection for hepatocellular carcinoma. Surgery 1990; 107:511–520.
45. Franco D, Capussotti L, Smadja C, et al. Resection of hepatocellular carcinomas: results in 72 European patients with cirrhosis. Gastroenterology 1990; 98:733–738.
46. Smalley SR, Moertel CG, Hilton JF, et al. Hepatoma in the noncirrhotic liver. Cancer 1988; 62:1414–1424.
47. Flatmark A, Frethem B, Knutrud O, Lande G. Surgical treatment of primary liver carcinoma. Scand J Gastroenterol 1977; 12:571–575.
48. Nagorney DM, van Heerden JA, Ilstrup DM, Adson MA. Primary hepatic malignancy: surgical management and determinants of survival. Surgery 1989; 106:740–749.
49. Adson MA, Weiland LH. Resection of primary solid hepatic tumors. Am J Surg 1981; 141:18–21.
50. Honjo I, Mizumoto R. Primary carcinoma of the liver. Am J Surg 1974; 128:31–36.
51. Pachter HL, Spencer FC, Hofstetter SR, Coppa GF. Experience with the finger fracture technique to achieve intra-hepatic hemostasis in 75 patients with severe injuries of the liver. Ann Surg 1983; 197:771–778.
52. Balasegaram M, Joishy SK. Hepatic resection: the logical approach to surgical management of major trauma to the liver. Am J Surg 1981; 142:580–583.
53. Feliciano DV. Surgery for liver trauma. Surg Clin North Am 1989; 69:273–284.
54. Pace RF, Blenkharn JI, Edwards WJ, et al. Intra-abdominal sepsis after hepatic resection. Ann Surg 1989; 209:302–306.
55. Lucas CE, Ledgerwood AM. Prospective evaluation of hemostatic techniques for liver injuries. J Trauma 1976; 16:442–451.
56. Pachter HL, Spencer FC. Recent concepts in the treatment of hepatic trauma. Ann Surg 1979; 190:423–429.
57. Ryan WH, Hummel BW, McClelland RN. Reduction in the morbidity and mortality of major hepatic resection: experience with 52 patients. Am J Surg 1982; 144:740–743.
58. Stimpson REJ, Pellegrini CA, Way LW. Factors affecting the morbidity of elective liver resection. Am J Surg 1987; 153:189–196.
59. Starzl TE, Bell RH, Beart RW, Putnam CW. Hepatic trisegmentectomy and other liver resections. Surg Gynecol Obstet 1975; 141: 429–437.
60. Bismuth H, Houssin D, Michel F. Le risque opératoire des hépatectomies: expérience sur 154 hépatectomies. Chirugie 1983; 109: 342–348.
61. Iwatsuki S, Shaw BW, Starzl TE. Experience with 150 liver resections. Ann Surg 1983; 197:247–253.
62. Thompson HH, Tompkins RK, Longmire WP Jr. Major hepatic resection: a 25-year experience. Ann Surg 1983; 197:375–388.
63. Iwatsuki S, Starzl TE. Personal experience with 411 hepatic resections. Ann Surg 1988; 208:421–434.
64. Balasegaram M, Joishy SK. Hepatic resection: pillars of success built on the foundation of 15 years of experience. Am J Surg 1981; 141:360–365.
65. Foster JH. Survival after liver resection for secondary tumors. Am J Surg 1978; 135:389–394.
66. Flanagan L, Foster JH. Hepatic resection for metastatic cancer. Am J Surg 1967; 113:551–557.
67. Brown DA, Pommier RF, Woltering EA, Fletcher WS. Nonanatomic hepatic resection for secondary hepatic tumors with special reference to hemostatic technique. Arch Surg 1988; 123:106:3–6.

Liver Transplantation in Patients With Portal Vein Thrombosis and Central Portacaval Shunts

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The authors have analyzed the impact of pre-existing portal vein pathology on the outcome of orthotopic liver transplantation. The incidence was high in patients suffering from chronic active hepatitis, hypercoagulable states, trauma or previous dissection of the porta hepatis, and splenectomy. The existence of portal vein thrombosis (23 patients) or surgical central portosystemic shunt (10 patients) was documented by preoperative Doppler sonogram or angiography (26/33), or operative findings of occluded vein (7/33). Successful thrombectomy and dismantling of portacaval shunts were achieved in most cases (24/33). Only nine patients required the placement of an interposition vein graft to the superior mesenteric vein. The intraoperative course was characterized by increased blood loss and coagulopathy, significantly higher than in patients with a patent portal vein. When compared with all liver transplants, the immediate postoperative complication rate was higher for primary nonfunction (33% *versus* 8%), re-exploration for intraperitoneal bleeding and hematomas, and morbid infections. Rethrombosis rate of thrombectomized veins or vein graft was low (2/33). The mortality rate was 35% in the presence of portal vein thrombosis (PVT) and 30% for portacaval shuct (PCS), both significantly higher than the 12% for other orthotopic liver transplant (OLT) patients. These results are expected to improve with better patient selection, surgical experience, and anticipation of the complex postoperative course. The authors conclude that PVT or the presence of PCS are not contraindications to orthotopic liver transplantation.

PRE-EXISTING PATHOLOGY OF the portal vein increases the surgical complexity of orthotopic liver transplantation (OLT) and may have a substantial impact on the perioperative morbidity and mortality rates.[1,2] Such abnormalities are defined as the preoperative or intraoperative findings of portal vein thrombosis (PVT) or the presence of central portosystemic shunts (portacaval shunt, PCS). This group of patients is considered to represent a higher surgical risk, and knowledge of such pathology during the evaluation process weighs on the decision of acceptance for OLT. In this study we describe evaluation, operative aspects, and the immediate and late postoperative complications of preoperative portal vein pathology. The purpose of this report is (1) to determine the impact of pre-existing portal vein pathology on the surgical procedure, (2) to identify specific preventable perioperative complications, (3) to describe reconstructive modalities for portal revascularization, and (4) based on our results, to determine whether this group of patients with end-stage liver disease are acceptable candidates for OLT.

Materials and Methods

The charts of 550 consecutive patients undergoing OLT at UCLA between 1986 and 1990 were examined. The patient population included 364 adults and 186 children, who underwent a total of 676 OLTs. Patency of the portal vein was determined in each case by ultrasound examination until 1986, and duplex sonogram since then. The findings of possible portal vein occlusion or the knowledge of previous portosystemic shunt were indications for selective celiac and superior mesenteric artery angiograms with plain and subtraction views of the venous phase. More recently magnetic resonance imaging or hemodynamic labeled ammonia nuclear scan were used in a few pediatric and adult patients. Information about the donor and recipient liver venous anatomy is detailed in operative fact sheets from both the donor and recipient operations and are available for all OLTs performed at UCLA. The method for portal vein reconstruction, including the exact anastomotic site between the recipient and the donor vein, successful performance of portal vein thrombectomy, and the placement of allogeneic iliac vein graft, was documented in each case. Postoperative follow-up included

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routine evaluation of portal vein patency, which was assessed in all patients by serial Doppler sonograms (duplex) studies and, if questionable, by selective venous phase angiography. Operative reports of redo cases and autopsy findings of early and late deaths were reviewed to determine the patency of the portal vein at the time of re-exploration or death. The overall intensive care unit (ICU) and hospital stay and complications directly related to the portal vein reconstruction were considered as important variables contributing to postoperative morbidity.

Operative Reconstruction of the Portal Vein

Techniques of recipient hepatectomy and grafting were employed as was previously described.[3] After dissection of the porta hepatis and assessment of the portal vein status, the patient was put on partial venous–venous bypass by cannulating the femoral and axillary veins.

Thrombosis of the portal vein was approached by dissection of its entire length to the confluence of the splenic and superior mesenteric veins (SMV). The portal vein then was incised transversely just inferior to its bifurcation, and thrombectomy was accomplished using endarterectomy spatula, Cannon strippers, or Fogarty balloon catheters. Successful removal of the thrombus, which was organized in most cases, allowed the insertion of a portal vein cannula and placement of the patient on full bypass (venous inflow through the portal and the iliac veins). In three cases, the portal cannula was not inserted because of fragility of the vein, or failure to detect intestinal congestion. Such patients were left on partial venous bypass; however, the thrombectomized vein was used as a conduit for portal blood inflow to the liver.

Failed thrombectomy or an absence of the portal vein were managed by placement of a donor iliac vein graft between the recipient SMV and donor portal vein. The vein graft was placed immediately after performance of the recipient hepatectomy and before grafting. During this period the patient was placed on full bypass using the inferior mesenteric vein (IMV) for portal inflow. Cannulas were never inserted into the vein graft.

TABLE 1. *Incidence of Pre-existing Portal Vein Pathology*

	Portal Vein Thrombosis	Portocaval Shunt
Adult patients (n = 28)		
Chronic active hepatitis	14	6
Primary biliary cirrhosis		2
Familial cholestasis	1	
Alcoholic cirrhosis	1	1
Budd–Chiari syndrome		1
Trauma	2	
Pediatric patients (n = 5)		
Biliary atresia	3	
α-1 antitrypsin deficiency	1	
Trauma	1	

TABLE 2. *Factors Associated With the Development of Portal Vein Thrombosis*

Factor	No.
Hematologic abnormalities (n = 3)	
Antithrombin III	1
Sickle cell	1
Polycythemia vera	1
Upper abdominal surgery (n = 14)	
Splenectomy	3
Trauma/iatrogenic/previous orthotopic liver transplantation	4
Kasai portoenterostomy	3
Cholecystectomy and bile duct exploration	4
Tumor thrombus (n = 2)	2

Dismantling portacaval shunts was performed as previously described by our group.[2] The length of the recipient portal vein and its patency determined the need for interposition of an iliac vein graft. It was possible to insert a portal vein cannula and place the patient on full bypass in most of these patients.

Rethrombosis of a thrombectomized portal vein was managed by placement of iliac vein graft to the SMV.

Results

Incidence of PVT and PCS

Pre-existing pathology of the portal vein was found over a wide spectrum of liver disease (Table 1). Of the 28 adult patients, the incidence of portal vein thrombosis or portacaval shunts was highest among those suffering from chronic active hepatitis (CAH, 20/28 patients, or 71%). This finding is different than the distribution of CAH, which encompasses 45% of all adult liver transplants. In contrast the portal vein was found to be patent in all cases of primary biliary cirrhosis and sclerosing cholangitis (22.5% of all adult liver transplants). In the pediatric population, the portal vein was thrombosed with cavernous transformation in three pediatric patients suffering from biliary atresia and in one child with α-1 antitrypsin deficiency.

A total of 23 patients were found to have an occluded or absent portal vein. Factors directly implicated in the development of PVT were found in 10 of 23 patients (Table 2). These patients were found to suffer from a hypercoagulable state (3/10), tumor thrombus (2/10), or have had previous surgery or trauma to the portal vein (5/10). In addition nine other patients had had upper abdominal operations that could have associated factors in the development of portal vein thrombosis. Three of these patients underwent splenectomy for symptomatic hypersplenism, the others underwent surgical exploration and extensive dissection around the porta hepatis (*e.g.*, Kasai portoenterostomy, cholecystectomy with common bile duct exploration).

Ten patients underwent portacaval shunt procedures at various times before OLT. The shunt was done in patients with recurrent variceal bleeding, preserved synthetic liver function, and no other major complications related to their liver disease.

Indications for OLT

All the patients were found to suffer from progressive end-stage liver disease, with complications that could not be controlled by more conservative management. There was a higher incidence of profound encephalopathy in the thrombosed or shunted group (70% and 80%, respectively) when compared with other OLT candidates (40%). In addition severe portal hypertension was found in all patients with PVT, but not in the presence of patent PCS. The existence of hepatocellular carcinoma limited to the liver was known in three patients before surgery, and in two patients tumor involved the bifurcation of the right and left portal branches.

Patency of the Portal Vein

A routine sonogram or Doppler sonogram (duplex) test is part of the preoperative assessment for all OLT candidates in our program. Pre-existing portal vein pathology such as previous portacaval shunt, or occlusion as demonstrated by sonogram, mandates a selective visceral angiogram. Flow through the splenic vein and the SMV were documented on the venous phase of selective splenic and SMA injections. Studies were not done in three patients known to suffer from recent trauma and portal vein ligation.

Duplex scan suggested an occlusion of the portal vein in 13 patients; these findings were confirmed by a selective celiac and SMA angiogram in 13 of 13 cases (Fig. 1). The duplex scan failed to detect an occluded vein in seven patients who were found to have this pathology during exploration and hepatectomy. Considering the large number of patients who underwent duplex scan, the specificity of this examination is >93% (open portal vein was confirmed in 510/550 patients). False-negative results were high, however, because the duplex failed to detect the presence of thrombosed vein, which was first seen during hepatectomy in 7 of 20 patients. Recent data suggest that the use of color duplex may have the same or higher specificity, but lower rate of false-negative results.[4]

Angiography was found to be a valuable and accurate test for patients who underwent various types of PCS, and those with sonographic evidence of an occluded portal vein. The portal vein was found to be open, and the PCS was patent in 10 of 10 patients (Fig. 1). In addition angiography confirmed the duplex findings of occluded portal vein in 10 of 10 patients. Moreover selective angiography helps to exclude all false-positive duplex findings, in other words, when the PV was thought to be occluded by sonographic test but was found open by the angiogram (data not shown).

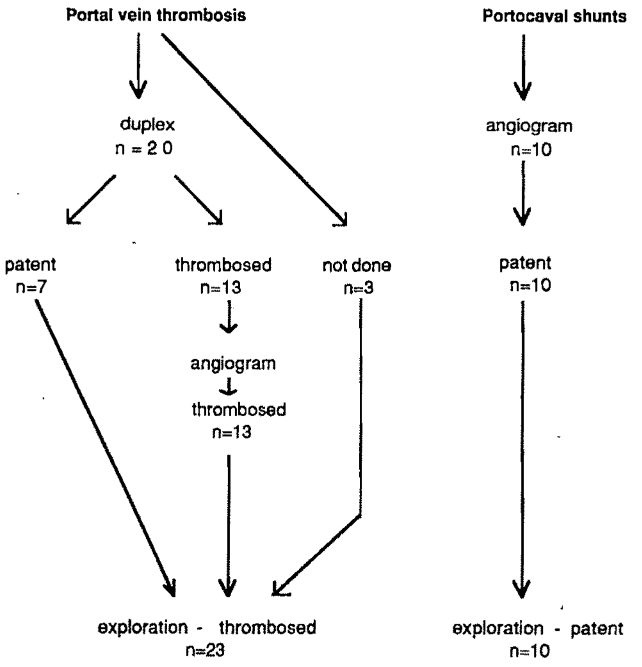

FIG. 1. Selective superior mesenteric artery angiography documented patent portal vein in the presence of portocaval shunt. Duplex scan was effective in the screening of most of the patients with portal vein thrombosis. However, occluded portal vein was found during exploration and hepatectomy in 7 of 23 cases.

Operative Management

The portal vein was successfully thrombectomized and preserved for anastomosis without the need for vein graft in 14 of 23 PVTs (Table 3). The coronary vein or large hepatoduodenal varix was used for portal inflow in two cases. Only seven patients needed the placement of donor iliac vein graft, six of seven were interposed to the SMV, and one of seven to the PV at the confluence of the splenic and the SMV.

The PCS was dismantled before the completion of the recipient hepatectomy in 10 of 10 patients (Table 3). In this group the entire length of the reconstructed vein was used as an inflow tract for portal blood perfusion of the liver graft.

Partial or full venous bypass was used in all the adult patients (Table 3). The full venous bypass was used in 17 of 33 patients, whereas partial bypass was used in 12 of 33 patients. Venous bypass was not used in four pediatric patients who were found to tolerate complete inferior vena cava (IVC) occlusion without significant changes in blood pressure or cardiac output.

TABLE 3. *Methods of Portal Vein Reconstruction*

Method	Portal Vein Thrombosis (n = 23)	Portocaval Shunt (n = 10)
Repair		
Dismantling with primary repair		10
Thrombectomy	14	
Vein graft		
to portal vein	1	
to superior mesenteric vein	6	
to coronary vein	2	
Venous bypass		
Full bypass		
Portal vein cannula	11	6
Inferior mesenteric vein cannula	3	
Partial bypass	7	4

TABLE 4. *Intraoperative Blood Loss and Urine Output*

	Portal Vein Thrombosis (n = 23)	Portocaval Shunt (n = 10)
Adult patients (n = 28)		
Estimated blood loss (blood volumes, mean + SD)	10.5 ± 10.2	12.4 ± 9.7
Urine output (ml/hr)	126	85
Pediatric patients (n = 5)		
Estimated blood loss (blood volumes, mean + SD)	9.1 ± 6.3	
Urine output (ml/kg/hr)	26	

Successful removal of the adherent clot and thrombus allowed the insertion of a portal vein cannula with the use of full venous–venous bypass in 11 of 14 patients with PVT. Flow rates through the thrombectomized vein exceeded 1000 cc/min. Patients who needed a portal vein graft were put on partial bypass (7/9) or full bypass using the IMV for portal blood outflow tract (3/9). Flow rates through the IMV were above 750 cc/min. It was elected to insert a portal cannula for full venous bypass in only 6 of 10 PCS cases. This was not done in the other four patients to minimize the trauma to an extremely delicate portal vein.

Fourteen patients required retransplantation at some point. The previously thrombectomized portal veins or portal vein grafts were successfully reused in all but two cases. Only one adult patient rethrombosed his portal vein, and portal blood flow to the new graft was established with the placement of interposition vein graft to SMV. The other patient, a child, clotted a vein graft to the SMV and died of septic complications before retransplantation. Venous–venous bypass was used in the retransplanted group with the same success rate as for the first transplant. Because all but one patient were found to have an open portal vein, the portal system was decompressed in the same fashion as the first OLT. The portal system of the patient with the thrombosed vein was decompressed through the IMV.

Intraoperative Complications

All patients maintained hemodynamic stability during their procedure. There was a significant increase, however, in the requirement of blood transfusion and the development of coagulopathy for transplanted patients with PCS and PVT (Table 4). A mean of 10.5 blood volumes were used for each adult patient. This is significantly higher than our average of blood loss of 3.2 blood volumes for all adult OLT cases. Correction of the coagulopathy required the continuous transfusion of fresh frozen plasma, as well as platelets and cryoprecipitate. These results were found to be similar in the pediatric patients. The mean blood transfusion was 9.1 blood volume, substantially higher then the average 3.3 blood volume in all pediatric OLT patients. Despite the excessive blood loss during procedure, most of the patients were kept with stable blood pressure. Urine output was >1 cc/kg/hr, and only four patients needed intraoperative or immediate postoperative dialysis (Table 4). There were no intraoperative deaths in this series.

Liver-related Postoperative Complications

Primary Nonfunction. The incidence of early (immediate) or delayed (3 to 6 days) primary nonfunction (PNF) of the liver graft was 35% for PVT (8/23) and 40% for PCS (4/10) (Table 5). These results are in contrast to the overall 8% incidence of PNF in our total experience with 676 liver transplants. Primary nonfunction was more common in patients with blood transfusion exceeding 60 units of packed red blood cells (PRBC). Reviewing the donor data and harvesting operative notes revealed three very unstable donors. These livers were used because of the urgent needs of the recipients.

Hepatic Artery Thrombosis. The incidence of hepatic artery thrombosis (HAT) was 9% (3/33) and occurred in two adult and one pediatric patient. These patients were out of the ICU with excellent graft function at the time

TABLE 5. *Postoperative Graft Complications*

	Portal Vein Thrombosis (n = 23)	Portocaval Shunt (n = 10)
Total retransplants (n = 15)		
Primary nonfunction	3	2
Delayed nonfunction (3–7 days)	4	2
Vascular complications (n = 4)		
Hepatic artery thrombosis	3	
Portal vein thrombosis	2	
Exploratory laparotomy (n = 14)		
Intra-abdominal bleeding	7	3
Bile leak	4	

of thrombosis. Hepatic artery thrombosis was attributed to hypotension after upper gastrointestinal bleeding in one pediatric patient; no specific cause was identified in the other two.

PVT. Rethrombosis of the portal vein occurred in 2 of 33 patients. In one case a thrombectomized vein was found to be occluded at the time of exploration for retransplantation. The clinical presentation was delayed PNF of the graft. The pathophysiology leading to rethrombosis can be explained, in retrospect, by poor venous blood flow through the thrombectomized vein during the first OLT (In this case the portal vein was not cannulated for venous bypass during the first OLT). The second patient had had an interposition vein graft to the SMV; the underlying pathology for occlusion of the graft was never found. The clinical presentation was progressive graft failure and severe sepsis.

Intra-abdominal Bleeding. Re-exploration for continuous intra-abdominal bleeding was required in 10 of 33 patients. The source of bleeding was not found in any of these patients, and was probably related to some degree of coagulopathy after multiple blood transfusions, as well as to poor synthetic function of the liver graft.

Bile Leak. Disrupted bile duct anastomosis was found in four patients, three of whom had HAT, and one case of portal vein rethrombosis. All three patients with HAT died of sepsis despite retransplantation.

Hospital Stay, Morbidity Rate, and Mortality Rate

Patients with pre-existing portal vein pathology were found to have prolonged ICU and hospital admission after OLT (Table 6). The survivors stayed an average of 11.5 days (PVT), and 16 days (PCS) in the ICU, with a total of 53.9 and 50 hospital days before discharge, respectively. This is in contrast to 6 ICU and 41.9 hospital days for all adult liver transplant patients.

Significant renal failure was seen in 4 of 22 survivors who needed prolonged postoperative dialysis. Kidney function returned to normal in all three cases, and compared favorably with other liver transplanted patients. Severe cytomegalovirus infection occurred in 2 of 22 survivors, which resolved after treatment with DHPG (ganciclovir).

The three-month mortality rates were 35% for the PVT and 30% for PCS patients. These are higher than the 12% 3-month mortality rate for all OLT at UCLA. The nonsurviving patients (11/33) stayed in the ICU for most of their hospital admissions. The cause of death was related to the development of uncontrolled fatal infections in 9 of 11 patients. The source of infection was related to peritonitis from bile leak (3/9), bowel perforation (1/9), pneumonia (3/9), and systemic infection from unknown source (2/9). In addition two patients died of rapidly progressive graft failure. These patients were comatose with evidence of irreversible brain damage, hemodynamically unstable and septic, and were not considered candidates for retransplantation.

Discussion

Patients with end-stage liver disease and documented thrombosed portal vein or prior portacaval shunt are considered high-risk patients, and are not uncommonly rejected as potential candidates, or referred to more experienced transplant centers. This approach to these patients is based on the complexity of portal vein reconstruction, and the clinical impression of increased morbidity and mortality rates in this group.[1,2] In this report we describe our results with OLT in the presence of pre-existing portal vein pathology.

The necessity for portal blood flow perfusion to the liver graft is well documented in early studies by Marchioro et al.[5] and Starzl et al.[6] Thus the presence of pre-existing portal vein pathology and its potentially deleterious effects on the surgical procedure and graft survival has lead us to a policy of mandatory preoperative documentation of portal vein patency before OLT. The information was helpful to the medical and surgical team in the selection and preparation of the patient for a more complex procedure. The most common noninvasive reliable method is a duplex scan, which is accurate in identifying the vascular anatomy in 93% of the patients.[7,8] The small number of false-positive results were all eliminated by selective splanchnic angiography. The duplex scan test failed to detect PVT in a few patients found to have thrombosis during the hepatectomy. Other noninvasive tests to be considered are color duplex scan, magnetic resonance imaging, or nuclear imaging techniques.[9,10] We are studying the sensitivity of the color duplex scan in the detection of arterial and venous blood flow in the transplanted liver. Our preliminary results, as well as studies in peripheral vascular diseases, suggest that

TABLE 6. *Hospital and Intensive Care Unit Stay*

Duration of admission (mean + SD)	Portal Vein Thrombosis		Portocaval Shunt	
	Alive (n = 15)	Dead (n = 8)	Alive (n = 7)	Dead (n = 3)
Intensive care unit	11.5 (9.4)	41.4 (28)	16.0 (18.2)	24.4 (15.7)
Hospital	46.7 (25)	41.7 (27)	50.0 (21.2)	74.0 (45.6)

this test is more accurate in the detection of thrombi in the venous system.[4] Although selective celiac and mesenteric angiography was found to be an extremely reliable test, the use of this invasive and expensive procedure should be reserved for patients who are believed to have pre-existing portal vein pathology by duplex scan or a known surgical portosystemic shunt. In these patients it is important to determine the exact anatomy of the portal system by selective angiogram, because complete absence of a patent SMV or major mesenteric branches suitable to serve as a portal blood outflow tract are a contraindication to OLT.

The overall occurrence of PVT and PCS was found to be 6% (33/550 patients). To identify specific populations who are more prone to develop PVT, we have analyzed the incidence of this pathology in various types of end-stage liver diseases. A higher occurrence of PVT was found in patients suffering from chronic active hepatitis (CAH). Thrombosis of the portal vein was not seen in any patient with end-stage primary biliary cirrhosis or sclerosing cholangitis. This observation may relate to the impedance to blood flow through a small shrunken liver in patients with CAH. Thrombosis also was seen in patients with various hematologic disorders (*e.g.* polycythemia vera, Budd–Chiari, antithrombin III deficiency, etc.), as well as in patients who had a previous splenectomy or upper abdominal surgery involving dissection of the porta hepatis. Currently we document the patency of the portal vein by color duplex scan in these groups, or in equivocal cases we use angiography.

The operative reconstruction of the portal vein has been reported in several small series, as well as in a few case reports. It was shown that the occluded portal vein can be thrombectomized or bypassed.[11–13] The option of thrombectomy was exercised successfully in most of our patients, and was found to be safe, with a low re-thrombosis rate. Unlike previous reports, we were able to use the thrombectomized portal vein as a source of splanchnic outflow during venovenous bypass without the propagation of pulmonary embolus though the bypass system.[1] In the presence of surgical portacaval shunt, the shunt can be dismantled,[2] and the whole length of the vein can be reconstructed and used for successful portal blood revascularization of the graft. Once the shunt is taken down, it is safe to insert a portal cannula and place the patient on full venous–venous bypass.

When the native portal vein is not suitable for reconstruction, a donor iliac vein graft can be interposed between the recipient SMV and the donor portal vein.[14,15] The dissection in the colonic mesentery below the pancreas to locate the SMV is similar to that used for mesocaval shunts. Long-term follow-up in a few case reports found the grafts to stay patent. Using this procedure we encountered only one case of graft thrombosis. For the protection of the venous anastomosis, a portal cannula is not to be inserted through a vein graft to the SMV. In these cases, the portal system can be successfully decompressed through the IMV.[16]

As demonstrated in this study and other reports, the technical complexities of portal vein reconstruction can be solved. A different issue is whether these patients are acceptable candidates for OLT. Patients with PVT were rejected as OLT candidates in a few early series[17–19]; no specific indication is given for such exclusion. Because it is technically possible to reconstruct and restore portal blood supply to the liver graft, it is our opinion that this group of patients should be considered as OLT candidates. The increased risks should be understood and accepted, however, by the patients and the surgical team. Our results demonstrate that a pre-existing portal vein pathology is associated with increased perioperative complications and mortality rates. The intraoperative course is characterized by high blood loss, the need for multiple blood products, the development of coagulopathy, and increased rate of primary nonfunction of the new graft. This is not surprising, because it is expected that a difficult portal dissection in the presence of occluded portal vein and severe portal hypertension result in significant bleeding during hepatectomy. This is also true for patients with surgical portacaval shunt because the dissection of the shunt in scarred tissue planes often results in excessive bleeding. As a result, during the anhepatic phase, the patients develop severe acidosis and coagulopathy. The new liver graft is thus transplanted under less than optimal conditions in a compromised host, and the probabilities for failure are increased. We assume that this explains the relatively high rate of primary nonfunction of the graft, the prolonged postoperative coagulopathy, and the need for further explorations for evacuation of intra-abdominal hematomas. The possibility that sudden changes in the portal system or the prolonged period of diverted portal blood have some effect on the new graft can not be excluded.

The consequence of multiple explorations for intraperitoneal bleeding, bile leaks, or retransplantation in an immunocompromised host, and the prolonged ICU admission are the development of morbid infections and increased mortality rate.[20,21] The mortality rate in the presence of a thrombosed portal vein is 35%, and for portacaval shunt it is 30%. Collective review of small number of case reports show an early mortality rate of 15% to 50%.[11,14,15,22] These figures are significantly higher than the 86% 1-year survival for other OLT patients in our center. After a definite learning curve, however, our recent results during the past year are more encouraging. Anticipating a complicated course for patients with PVT or PCS allow better selection of the candidates. Patients with PVT or PCS are accepted as transplant candidates in the absence of other significant organ system failure. Preoperative findings of tumor thrombus obstructing the portal vein is considered as contraindication for OLT. We now limit cold ischemic time to 8 hours for these difficult cases, in the hope of reducing the rate of PNF. Postoperatively the patency of the portal vein is followed by a routine duplex scan. Early exploration for thrombosed vein or artery, removal of thrombus, and re-do of the anastomosis may result in salvage of the graft. The current success rate in this group and the absence of any other alternative treatment justify the acceptance of patients with pre-existing portal vein pathology as OLT candidates.

Addendum

Since the submission of this article, the authors have operated on four more patients with portocaval shunts and three patients with portal vein thrombosis. The increased surgical experience with this pre-existing pathology of the portal vein, and the concerted effort to shorten ischemic time, resulted in no retransplants, less morbidity, and 100% patient survival. Our overall survival rate, to date, in patients with portal vein pathology is 72.5%.

References

1. Lerut J, Tzakis AG, Bron K, et al. Complications of venous reconstruction in human orthotopic liver transplantation. Ann Surg 1987; 205:404–414.
2. Brems JJ, Hiatt JR, Busuttil RW, et al. Effect of prior portasystemic shunt on subsequent liver transplantation. Ann Surg 1989; 209: 51–56.
3. Busuttil RW, Colonna JO, Hiatt JR, et al. The first 100 liver transplant at UCLA. Ann Surg 1987; 206:387–402.
4. Grant EG, Tessler FN, Gomez AS, et al. Color Doppler imaging of portosystemic shunts. Am J Radiol 1990; 154:393–397.
5. Marchioro MA, Porter KA, Dickenson TC, et al. Physiologic requirement for auxiliary liver homotransplantation. Surg Gnnecol Obstet 1965; 21:17–21.
6. Starzl TE, Francavilla A, Halgrimson CG, et al. The origin, hormonal nature, and action of hepatotrophic substances in portal venous blood. Surg Gynecol Obstet 1973; 137:179–199.
7. Alpren MB, Rubin JM, Williams DM, Capek P. Portahepatis: Duplex doppler US with angiographic correlation. Radiology; 1987; 162: 53–56.
8. Raby N, Karani J, Powell-Jackson P, et al. Assessment of portal vein patency: comparison of arterial portography and ultra scanning. Clin Radiol 1988; 39:381–385.
9. Ralls PW. Color doppler sonography of the hepatic artery and portal venous system. Am J Roentgenol 1990; 155:517–525.
10. Bisset GS, Strife JL, Balistreri WF. Evaluation of children for liver transplantation: value of MR imaging and sonography. Am J Roentgenol 1990; 155:351–356.
11. Shaw BW, Iwatsuki S, Bron K. Portal vein grafts in hepatic transplantation. Surg Obstet Gynecol 1985; 161:66–68.
12. Tzakis A, Todo S, Stieber A, Starzl TE. Venous jump graft for liver transplantation in patients with portal vein thrombosis. Transplantation 1989; 48:530–531.
13. Haitt JR, Quinones-Baldrich WJ, Ramming KP, et al. Bile duct varices: an alternative to porto-portal anastomosis in liver transplantation. Transplantation 1986; 42:85.
14. Kirsh JP, Howard TK, Klintmalm GB, et al. Problematic vascular reconstruction in liver transplantation part II. Portovenous conduits. Surgery 1990; 107:544–548.
15. Burdick JF, Pitt HA, Colombani PM, et al. Superior mesenteric inflow for liver transplantation when the portal vein is occluded. Surgery 1990; 107:342–345.
16. Slooff MJH, Bams WJ, Sluiter IJ, et al. A modified cannulation technique for veno-venous bypass during orthotopic liver transplantation. Transplant Proc 1989; 21(1 pt 2):2328–2329.
17. Jenkins RL, Benotti PN, Bothe AA, Rossi RL. Liver transplantation. Surg Clin North Am 1985; 65:103–122.
18. Van Der Putten ABMM, Bijleveld CMA, Slooff MJH, et al. Selection criteria and decisions in 375 patients with liver disease, considered for liver transplantation during 1977–1985. Liver 1987; 7:84–90.
19. Van Thiel PH, Schade RR, Starzl TE, et al. Liver transplantation in adults. Hepatology 1982; 2:637–640.
20. Colonna JO, Winston DJ, Brill JE, et al. Infectious complications in liver transplantation. Arch Surg 1988; 123:360–364.
21. Castaldo P, Stratta RJ, Wood P, et al. Clinical spectrum of fungal infections after orthotopic liver transplantation. Arch Surg 1991; 126:149–156.
22. Williams JW, Britt LG, Peters TG, et al. Portal vein obstruction in patients requiring hepatic resection or transplantation. Am Surg 1984; 50:365–468.

Experience With 100 Consecutive Simultaneous Kidney–Pancreas Transplants With Bladder Drainage

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From December 1985 to December 1989, 100 consecutive simultaneous pancreas–kidney (SPK) transplants were performed at the University of Wisconsin Hospital and Clinics. Bladder drainage technique was used for all grafts. One- and three-year patient survival was 93% and 90%; kidney survival, 90% and 85%; and pancreas survival, 86% and 84%. Quadruple immunosuppressive therapy was used in all patients and consisted of either MALG or OKT3 induction, and cyclosporine, prednisone, and azathioprine maintenance therapy. OKT3 induction therapy was used in 42 patients and Minnesota antilymphocyte globul in 58 patients, with equivalent results except for more opportunistic infection with OKT3. The duodenal button technique was used in the first 17 patients with systemic anticoagulation and was associated with significantly more postoperative bleeding and infection compared with the duodenal segment technique. The most common surgical complication of the duodenal segment technique was urine leak (13%), which usually occurred from the distal duodenal segment. The most common urologic complication of bladder drainage was hematuria (13%), which was usually self-limited. Within 6 months of transplantation, 84% of patients developed urinary tract infections, and 25% of patients had opportunistic infections. The surgical techniques and immunosuppressive methods used in this series are reviewed, and strategies for preventing medical and surgical complications are outlined.

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FROM DECEMBER 1966 TO December 1990, 2016 pancreas transplants were reported to the International Pancreas Transplant Registry. The functional survival rate was significantly higher for bladder (58%, n = 1566) than for either intestinal-drained (50%, n = 323, $p < 0.001$) or duct-injected grafts (43%, n = 174, $p < 0.001$; personal communication, Kay Moudry-Munns, R.N., University of Minnesota, International Pancreas Transplant Registry. Data reported and analyzed from January 1, 1986, to October 31, 1990) at 3 years' follow-up. From October 1, 1987 through June 30, 1989, 343 pancreas transplants were performed in the United States.[1] All but 10 of them employed the urinary drainage technique, with a 1-year patient survival of 91% and a 1-year graft survival rate for those pancreatic grafts transplanted simultaneously with a kidney (n = 236) of 75%. Although these data attest to the widespread and successful application of urinary drainage in pancreas transplantation, the procedure is still associated with significant problems and occasional life-threatening complications. The purpose of this paper is to present our series of 100 consecutively performed SPK transplants, to describe modifications in technique and immunosuppressive protocols, and to outline problems and complications as well as strategies to avoid them.

Materials and Methods

Patients

Patient characteristics are shown in Table 1. To qualify for simultaneous pancreas–kidney (SPK) transplantation at University of Wisconsin Hospital and Clinics, all patients more than 30 years of age had to undergo a thallium stress test.[2] If this test was suggestive of coronary artery disease, coronary angiograms were requested. Only patients with no or minimal coronary artery disease were accepted for SPK transplantation. Other contraindications to SPK transplantation included amputations due to atherosclerotic vascular disease, blindness, age above 55, and the inability to understand the investigational nature of the procedure, as well as the inability to comply with the extensive follow-up program. All patients were offered the opportunity to participate in a study investigating the effect of SPK transplantation on diabetic eye disease and in a comprehensive quality of life evaluation.

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TABLE 1. *Patient Characteristics of 100 Patients Undergoing Simultaneous Pancreas–Kidney Transplantation*

Characteristic	
Mean age (range)	35 yr (21–35)
Mean duration of diabetes (range)	23 yr (9–40)
Sex	
M	61%
F	39%
Transfused	83%
Dialysis	47%
Hypertension	90%
Retinopathy	93%
Neuropathy	47%
Amputation	3%

Organ Procurement and Preservation

Techniques and procedures used in organ procurement and preservation were previously described in detail.[3,4] Donor age ranged from 4 to 51 years. Eighty-two donors were dopamine dependent. Ninety-five grafts were preserved in University of Wisconsin (UW) solution (Viaspan; DuPont, Wilmington, DE) after intra-aortic *in situ* flush with UW solution. Five grafts were preserved in Collins solution. Mean preservation time for pancreatic grafts preserved in UW solution was 16.9 hours (range, 4 to 27.5 hours). Mean preservation time for kidneys (cold stored in UW solution) was 18.1 hours (range, 5.3 to 28.7 hours). In 64 donors the liver and pancreas were recovered simultaneously using a method described previously.[4] Before the procurement procedure, 250 mL of amphotericin B solution (50 mg/L H_2O) and 250 mL 1% povidone iodine solution were instilled into the stomach through a nasogastric tube. During procurement the administration of crystalloids was kept to a minimum and volume was preferentially replaced with colloids and blood. Before transplantation of the pancreas, duodenal contents were aspirated and sent for culture.

Immunosuppression

Forty-two patients were treated with antilymphocyte globulin induction protocol. The patients received 120 mg of methylprednisolone (Solu Medrol®; Upjohn, Kalamazoo, MI) intravenously (I.V.), 300 mg azathioprine (Quad, Indianapolis, IN) I.V., and 8 mg/kg cyclosporine A (Sandimmune, Sandoz Pharmaceuticals, East Hanover, NJ) by mouth before transplantation. Intraoperatively all patients received 500 mg methylprednisolone I.V. Minnesota antilymphocyte globulin (MALG) at a dose of 20 mg/kg I.V. was started on the first or second postoperative day and was continued until a therapeutic cyclosporine A plasma level was achieved under oral dosing. Dose adjustments were made depending on peripheral white cell count and platelets. Methylprednisolone was given intravenously for 3 days postoperatively at a dose of 120 mg/day and tapered within 1 week to 30 mg. Azathioprine maintenance dose ranged from 1 to 2 mg/kg/day and was adjusted to maintain a white cell count above 4000/mm^3. Cyclosporine was started when the serum creatinine level was less than 3, and the dose was adjusted to maintain a serum level of 350 to 500 ng/L (whole blood radioimmunoassay).

Fifty-eight patients were treated with an OKT3 (Orthoclone OKT3, Ortho Pharmaceuticals, Raritan, NJ) induction protocol. Azathioprine, steroids, and cyclosporine were administered in an identical manner as described in the MALG induction protocol. Instead of MALG, OKT3 was given at a dose of 5 mg starting intraoperatively at the time of the anastomosis and continued for 10 to 12 days after operation.

No standard protocol was used for the treatment of rejection episodes. In general mild rejections were treated with a short course of steroids (3 mg/kg/day for 3 days with a rapid taper to 30 mg/day). Severe rejections, as determined by renal biopsy, or rejections not responding to high-dose steroids were treated with a 10- to 14-day course of OKT3 (5 to 10 mg/day I.V.).

Operative Procedure

The operative procedure for the duodenal button (DB) technique was described previously in detail.[5,6] In 83 patients the duodenal segment (DS) technique first described by Nghiem and Bentel[7] was used. The whole pancreatic graft was anastomosed to the right iliac vessels using end-to-side anastomosis of the portal vein to the iliac vein. In 64 cases in which the liver and pancreas were procured from the same donor, an iliac artery bifurcation graft was anastomosed to the splenic artery and superior mesenteric artery. In the majority of cases, the common iliac artery was anastomosed to the superior mesenteric artery and the internal iliac artery was anastomosed to the splenic artery. Length of the portal vein was judged to be sufficient in all 100 cases, and no venous interposition graft was used. A 3- to 4-cm opening in the duodenum was made with electrocautery, and the margins were carefully electrocoagulated. An opening of identical size was made into the dome of the bladder, and pancreaticoduodenocystostomy was performed using two-layer suture technique using an outer interrupted layer of 3–0 Ethibond (Ethicon, Somerville, NJ) and a running layer with 4–0 polydiaxanone (PDS). In the past 19 cases, PDS was used for both layers. Although in the first 80 cases the spleen was left in place after completion of the anastomosis and splenectomy was performed before closing the abdomen, in the past 20 cases splenectomy was performed on the back table before performing the vascular anastomosis. In the last 93 cases, a long midline incision extending from the symphysis pubis to approximately 10 cm below the xiphoid was used. In seven early cases, a bilateral lower abdominal incision similar to the standard incision for a kidney transplant was used, and in all cases the pancreas was placed on the right side and the kidney was placed on the left side. In all cases the organs were placed intraperitoneally. The kidney was covered with sigmoid and sigmoid mesocolon. In 67 patients the pancreas was drained with two flat 10-mm Jackson-Pratt drains, which were brought out through two lateral stab wounds on the right side of the abdomen. In the last 33 cases, no drain was used.

Preoperative and Postoperative Medical Management

Preoperative antibiotics included 1 g cefazolin (Cefzol®, Lilly Co., Indianapolis, IN) I.V. and 1 g Vancomycin (Vancoled®, Lederle, Pearl River, NY) I.V. Dipyridamole (Cenera, Generic) 200 mg was administered by mouth, and aspirin (Uniseats®, Upsher-Smith, Minneapolis, MN) 650 mg by suppository. In women, miconazole vaginal cream 2% was applied to the perineal area. Postoperatively all patients received trimethoprim-sulfamethoxazole, double strength (TMP-sulfa DS), 1 tablet a day as maintenance therapy for the first year after transplant as soon as serum creatinine decreased to below 2.5 mg/dL. Clotrimazole troches (Monistat 7®; Ortho, Raritan, NJ), 10 mg four times a day, were administered for the first 3 months after transplant. Aspirin, 350 mg once daily, and dipyridamole, 75 mg four times a day, were administered from the first postoperative day and continued for at least 1 month after transplant. Ketoconazole tablets (Nizoral®, Janssen, Piscataway, NJ), 400 mg by mouth, and Vancomycin, 500 mg every 8 hours for 3 days were administered I.V. until the culture results of the duodenal aspirate were available. If the culture result was negative for bacteria or fungi, antibiotics were discontinued. In cases of positive cultures, antibiotic coverage was adjusted to the organism identified and continued for 10 days. All patients received acyclovir 200 mg by mouth four times a day for the first 3 months after transplant.

Infections were retrospectively analyzed and classified as previously described.[8] Cytomegalovirus (CMV) infection was defined by isolation of virus in a clinical specimen from any site or a fourfold rise in CMV antibody titer. Diagnosis of CMV disease required clinical symptoms compatible with CMV infection and isolation of the virus from a site other than blood or urine. In most cases, routine histologic evaluations of involved tissue were used to confirm the diagnosis; however, since 1988 rapid shell vial cultures also were used to diagnose CMV disease or infection.

Results

Initial Graft Function

Mean preoperative serum creatinine was 7.04 (±3.24) mg/dL. Mean serum creatinine on day 7 after transplant was 2.11 (±2.03) mg/dL and 1.75 (±0.6) mg/dL on discharge. Urine amylase rose from a mean value of 11,320 (±7281) μm/L on the fourth transplant day to 30,311 (±21,421) μm/L on day 10 after transplantation.

Reasons for Graft Failure

Graft loss for the pancreas was due to death with a functioning graft in six patients, rejection in five patients, pancreatitis in two patients, and thrombosis in two patients. Five patients died with functioning kidneys, and nine lost kidneys because of rejection. Time of death ranged from the 12th postoperative day to 30 months after transplantation. Two deaths were directly related to operative technical problems (rupture of mycotic iliac artery aneurysm after bladder leak, pulmonary sepsis after pancreatitis due to pancreatic duct occlusion). Three patients lost their kidneys from rejection but have a functioning pancreas. Two of these patients received a successful second renal transplant. One of the two patients who lost her pancreas because of thrombosis was immediately retransplanted with a second pancreas. Seven patients lost both grafts from rejection.

ALG Versus *OKT3 Induction Therapy*

Patient characteristics in the two groups were similar with respect to age, sex distribution, duration of diabetes, preoperative dialysis, and human leukocyte antigen (HLA) matching (Table 2). There was a slight preference for better HLA-AB matching in the OKT3 group, but this did not reach statistical significance (p = 0.075). Pancreas and renal graft and patient survival in ALG and OKT3 induction groups were not significantly different at 1 year after transplantation (Table 3). Median serum creatinine at discharge was 1.8 in both groups. The frequency of rejection within the first 14 days after transplantation was

TABLE 2. *Patient Characteristics in ALG and OKT3 Induction Groups*

	Induction Group		
Characteristic	ALG	OKT3	p
Age	36.4	35.4	0.48
Sex			
M	27	34	
F	15	24	0.26
Duration of diabetes (yr)	24.2	21.9	
Dialyzed before operation	22	31	0.57
HLA-AB match			0.92
0	19	15	
1	16	35	
2	7	8	0.075
HLA-DR match			
0	22	32	0.46
1	13	21	
2	0	4	
Not done	7	1	

TABLE 3. *ALG and OKT3 Induction Protocol: Patient and Graft Survival*

	Induction Group		
	ALG (n=42)	OKT3 (n=58)	
1-yr patient survival	91%	95%	NS
1-yr kidney survival	88%	91%	NS
1-yr pancreas survival	83%	88%	NS
Discharge serum creatinine level (mg/dL)	1.8	1.8	NS

TABLE 5. *Types of Opportunistic Infection After OKT3 or ALG Induction Therapy*

	Induction Group	
Infection	OKT3 (n=19)	ALG (n=6)
Cytomegalovirus infection	10	3
Cytomegalovirus disease	6	1
Epstein–Barr virus	2	—
Cryptococcus	1	—
Candida esophagitis	—	1
Varicella	—	1

40% with OKT3 and 45% with ALG (Table 4). The overall frequency of rejection was similar in both groups. The only significant difference was the incidence of opportunistic infections, which was higher in the OKT3 induction group (Table 5). Based on these results, ALG is now preferred for induction therapy at this institution.[9]

Kidney and Pancreas Function

At the meeting of the American Society of Transplant Surgeons in June 1986, Wahlberg et al.[9a] reported consistent 72-hour preservation of the canine pancreas with 100% immediate function. These experimental data stimulated us to use UW solution in clinical pancreas transplantation, and indeed, the first clinical application of UW solution came in January of 1987, when we first preserved a pancreas and a kidney. Since that time we have preserved more than 130 pancreas transplants in UW solution, and 95 of these grafts were used for SPK transplantation. With growing confidence in this solution, we have expanded our preservation times to accommodate time required for tissue typing and operating schedule, and we have been able to accept patients on our waiting list who need more than half a day to reach the University of Wisconsin Hospital. With the growing number of centers performing liver transplantation and with the increased number of liver transplants performed at the University of Wisconsin Hospital since 1984, it soon became obvious that there would be a shortage of pancreas transplants, since it was our policy to abandon pancreatic procurement if a liver recipient was identified either locally or nationally. Therefore since April 1988, combined recovery of pancreas and liver has been performed in all donors where both liver and pancreas seemed suitable for transplantation. As demonstrated in Table 6, regardless of the type of procurement (pancreas alone *versus* pancreas plus liver), there was no patient who required insulin in the immediate post-transplant period. There was one graft in each group that thrombosed within 36 hours after transplantation. In one instance thrombosis was due to a technical error, and in the second patient, for unknown reasons, both liver and pancreas failed within 3 days after transplant. The kidneys from the same donor demonstrated severe acute tubular necrosis (ATN) despite the fact that the donor was hemodynamically stable and had good renal function before the recovery procedure. Dialysis was required in one patient in each group, in one instance for fluid overload, and in the second patient for prolonged ATN. One-year function of kidney and pancreas in the two groups was identical, indicating that combined procurement of liver and pancreas does not have a detrimental effect on graft outcome. From a technical aspect, it seems favorable if one team removes both liver and pancreas *en bloc.* The separation of liver and pancreas *in situ* is time consuming and might delay heart and liver teams. The removal of pancreas and liver *en bloc,* however, can be accomplished in about the same

TABLE 4. *Rejection After Simultaneous Pancreas–Kidney Transplantation*

	Induction Group		
	OKT3 (n=55)	ALG (n=42)	
Total no. of rejections (range)	87 (1–4)	67 (1–5)	NS
Patients with rejection	51 (93%)	36 (86%)	NS
Rescue rejection with OKT3 or ALG	24 (47%)	20 (55%)	NS
Rejections during induction (first 14 days)	23 (40%)	19 (45%)	NS

TABLE 6. *Transplant-Related Complications in Pancreas Alone* vs. *Pancreas and Liver Recovery*

	Pancreas Alone (n=36)	Pancreas + Liver (n=64)
Insulin requirement	0	0
Thrombosis	1 (2.7%)	1 (1.5%)
Dialysis	1 (2.7%)	1 (1.5%)
Pancreatitis	1 (2.7%)	1 (1.5%)
Bleeding	4 (11.1%)	2 (3.1%)
Total	7 (19.4%)	5 (7.8%)
1-Yr function		
Kidney	90.9%	91.1%
Pancreas	86.3%	89.0%

time that is required to remove a liver graft, and the organs can be safely separated on the back table.

Surgical Complications

Surgical complications were defined as events that required surgical or invasive radiologic intervention or correction. Bladder leaks that healed spontaneously after insertion of a Foley catheter were not included. Complications, as listed in Table 7, were more frequent in the DB group; however, not all the complications listed there can be directly attributed to this technique. For instance all patients who had bilateral lower abdominal incisions were in the group with the DB technique, and because we believe this technique is more prone to wound complications, this complication again cannot be directly attributed to the use of the DB. Four patients among the seven who were anticoagulated with heparin and Dextran had to be re-explored for bleeding. Bleeding sites were small arterial vessels in the area of the pancreatic head and uncinate process. Causes for bleeding in the nonanticoagulated group were a slipped tie at the distal splenic artery and a ureteral blood vessel. Bladder leaks at the site of the pancreaticoduodenocystostomy were prevented by the use of the lateral umbilical ligaments, which were wrapped around the anastomosis. After this modification, most leaks in the DS group occurred at the mesenteric border of the distal staple line, and we now use one ligament to reinforce this staple line. Two leaks in the DS group occurred at the anterior aspect of the duodenal segment and were most likely associated with duodenal ulcers secondary to CMV infections.

Urologic Complications

The most frequent urologic complication was hematuria (Table 8). Although hematuria is a common occurrence at nearly any time after transplant, usually this is transient and self-limiting. We list here only patients who required cystoscopy, clot evacuation, and, if a bleeding point could be identified, who underwent cauterization of a bleeding source. In this series no patient required surgical exploration for hematuria. Ureteral stricture occurred with equal frequency in the DB group and the DS group. Five patients in the DS group who were treated for recurring urinary tract infections were cystoscoped, and either protruding, nonabsorbable suture or stones forming around the suture had to be removed. In the DS group, conversion to enteric pancreatic drainage was required in two patients for urethral disruption and in three patients for recurrent hematuria.

TABLE 7. *Surgical Complications After Simultaneous Pancreas–Kidney Transplantation*

	Treatment Group		
Complication	All SPK (n=100)	Button (n=17)	Duodenal Segment (n=83)
Bleeding episodes	8%	23.5%	4.8%
Bladder leak	13%	17.6%	12%
Abscess	5%	11.7%	3.6%
Thrombosis	2%	5.8%	1.2%
Small bowel obstruction	5%	11.7%	4.8%
Negative laparotomy	3%	17.6%	1.2%
Wound infection	5%		2.4%
Lymphocele	1%	5.8%	1.2%
Fascitis	1%		
Renal artery stenosis	1%		1.2%

SPK, simultaneous pancreas–kidney.

TABLE 8. *Urologic Complications After Simultaneous Pancreas–Kidney Transplantation*

	Treatment Group		
	All SPK (n=100)	Button (n=17)	Duodenal Segment (n=83)
Hematuria (episodes)	13%	11.7%	13.2%
Urethral stricture	5%	5.8%	4.8%
Urethritis	3%	5.8%	2.4%
Ureteral obstruction	2%	5.8%	1.2%
Stone/suture removal	4%	0	4.8%

SPK, simultaneous pancreas–kidney.

Infections

Ninety-eight recipients had sufficient follow-up for analysis of infection after transplantation. One patient died in the early postoperative period, leaving 97 patients who have been followed from 2 to 62 months. The first 42 patients received ALG induction therapy and included 17 patients with the duodenal button technique. Since May 1987 the duodenal segment technique has been the preferred surgical technique for the bladder anastomosis. The remaining 55 patients received OKT3 induction therapy, and all underwent the duodenal segment technique. Although not a randomized group, infection was arbitrarily analyzed with respect to the induction therapy used at the time of transplantation.

Infection was common after transplantation, with 67% of the OKT3-treated patients developing infection *versus* 42% of the ALG induction group (Table 9). Urinary tract infections were the major cause of infection and were most common after transplantation because of prolonged Foley catheter drainage. Subsequent urinary tract infections were most often due to neurogenic bladder or retained bladder suture or stones. Surgical infections were most common in the ALG induction group: however 8 of the 12 surgical infections in this group occurred in patients who underwent the duodenal button technique, which has been associated with a higher rate of surgical complications.[6]

The incidence of opportunistic infections was significantly higher in the OKT3 induction group, with 34% of the OKT3 induction group developing opportunistic infection *versus* 14% of the ALG group ($p < 0.05$) (Table 9). The incidence of rejection with both induction therapies was the same, as was the use of OKT3 or ALG for rescue rejection after steroid failure. The incidence of opportunistic infection after rescue therapy of rejection was higher in the OKT3 induction group, but did not reach statistical significance.

The types of opportunistic infection after OKT3 or ALG induction therapy are shown in Table 5. Cytomegalovirus infection and disease was more common in the OKT3 induction group, with the additional occurrence of two cases of Epstein–Barr virus lymphoproliferative disease. Despite the higher incidence of opportunistic infection in the OKT3 induction group, only one patient died after developing disseminated lymphoproliferative disease. The only other death attributable to infection was a patient who developed an infected hematoma in the early post-transplant period and subsequently developed unremitting pseudomonas pneumonia and sepsis.

The Effect of Duodenal Segment Bacteriology on Subsequent Infections

The culture results of 65 duodenal segments were available for analysis (Table 10). Thirty-five of the submitted specimens (53.8%) did not show any growth, whereas in 30 specimens, one or multiple bacteria or fungi were identified. The types of organisms and their frequency are listed in Table 10. Infectious complications in the culture-negative *versus* the culture-positive group did not differ. In particular, intra-abdominal and peripancreatic infections as well as wound infections were not more frequent in the culture-positive group. In four patients, urinary tract infection was diagnosed within the first 14 days after transplantation and the organism responsible for the urinary tract infection was identical to one of the organisms cultured from the duodenal segment. As shown in Table 11, the most frequent organism cultured from the duodenal segment was *Candida albicans,* followed by *Lactobacillus, Staphylococcus aureus,* and *Streptococcus viridans.*

TABLE 9. *Infections Within 6 Months After Simultaneous Pancreas–Kidney Transplantation With OKT3 or ALG Induction*

	Induction Group	
	OKT3 (n=55)	ALG (n=42)
Patients with infection	37 (67%)	26 (61%)
Total no. of infections (range)	92 (1–11)	104 (1–10)
Urinary tract infections	32	52
Surgical infections	6 (11%)	12 (28%)*
Opportunistic infection after rescue therapy	7 (29%)	2 (10%)
Opportunistic infection overall	19 (34)	6 (14%)*

* $p < 0.05$

TABLE 10. *Infectious Complications in Patients with Positive* vs. *Negative Duodenal Culture Findings*

	Culture Findings		
	Negative	Positive	
No. patients, total	35 (53.8%)	30 (46.2%)	
No. patients with infections	14 (40%)	13 (43.3%)	NS
Intra-abdominal	2 (5.7%)	1 (3.3%)	NS
Wound	1 (2.8%)	2 (6.6%)	NS
Urinary tract infection	13 (37.1%)	10 (33.3%)	NS

Hospital Stay and Readmissions After SPK Transplantation

A total of 2944 admission days were calculated for the initial hospital stay, resulting in an average hospital stay of 29.4 (range, 12 to 165) days. The most common causes for prolonged hospitalization were acute rejection and surgical complications (Table 12). Twenty-one patients were never readmitted, whereas 79 patients required one or several readmissions (range, 1 to 8). The total number of readmission days for the entire group (only readmissions to University of Wisconsin Hospital were calculated) was 2956 days. The most frequent reason for readmission was for the diagnosis and treatment of infections, followed

TABLE 11. *Organisms Cultured From Duodenal Segments of 65 Pancreatic Grafts*

Organism	n
Candida	14
Lactobacillus	9
Staphylococcus aureus	7
Streptococcus viridans	7
Escherichia coli	6
Enterobacter	5
Klebsiella	4
Torulopsis	3
Corynebacterium	3
Bacteroides	3
Bifidobacterium	2
Enterococcus	2
Acinetobacter	1
Beta-hemoytic streptococcus	1
Streptococcus intermedius	1
Proteus	1
Citrobacter	1
Serratia	1
H. parainfluenza	1
Bacillus sp.	1
Clostridium	1

TABLE 12. *Readmissions for 100 Patients who Underwent Simultaneous Pancreas–Kidney Transplants*

Reason for Readmission	n
Infection	57
Acute rejection	47
Nontransplanted-related problem	47
Urologic problems	26
Miscellaneous transplant-related problems	23
Cyclosporine toxicity	9
Chronic rejection	3
Vascular problems	1
Lymphocele	2
Multiple problems	23
Total	238

TABLE 13. *Readmissions for Infections in 100 Patients who Underwent Simultaneous Pancreas–Kidney Transplant*

Site	n
Urinary tract infections	24
Viral, site indeterminate	8
Sepsis	7
Intra-abdominal	6
Wound	5
Fever of unknown origin	5
Osteomyelitis	3
Pneumonia	2
Gastrointestinal tract	4
Other infections	3
Multiple infections	2
Skin	1
Varicella	1
Total	71

by acute rejection and non–transplant-related problems, which included aspects of general care such as control of hypertension, vascular surgery, foot care, eye surgery, and multiple medical problems. Among infections, the most common reason for readmission was urinary tract infection and viral infection of indeterminate site, and these are listed in Table 13. The total number of readmissions for infection, totalling 71, differs from the number of readmissions for infections listed in Table 11, because some of the infections were grouped under admissions for medical problems.

Graft and Patient Survival

One- and two-year patient and graft survival after SPK transplantation are listed in Table 14. Also listed are the graft survival data for diabetic patients who have received living-related or cadaver kidney transplants during this same period under similar immunosuppressive management and general medical treatment. As can be seen from the table, there is no difference between these groups as far as patient and graft survival are concerned.

Discussion

The growing number of pancreas transplants performed with bladder drainage indicates that most transplant centers have accepted this technique as their procedure of choice.[10] Although bladder drainage seems to have significant advantages over duct injection and enteric drainage, the frequency of surgical infectious complications remains significant, even in experienced centers. Clearly the procurement and preservation of the pancreatic graft is of paramount importance and to a large degree determines the success of the procedure. Since the introduction of UW solution, no primary nonfunction of kidney or pancreas was experienced in our series, and the incidence of transplant pancreatitis has been reduced, as determined by hyperamylasemia and the incidence of peripancreatic fluid collections. We also demonstrate in this series that combined recovery of liver and pancreas is safe for all organs, and excellent function can be achieved. A detailed analysis of livers procured in conjunction with the pancreas was previously published.[4]

Patient selection is an important aspect of every pancreas transplant program, and sound clinical judgment has to be used in the selection of appropriate patients for SPK transplantation. In our view patients with advanced secondary complications such as blindness, severe coronary artery disease, and severe peripheral vascular disease are not only high-risk candidates for surgery, but their complications are too far advanced to expect a beneficial effect of a functioning pancreas graft. We only consider

TABLE 14. *Actuarial Patient and Graft Survival in Diabetic Patients**

Length of Survival (yr)	% Survival
Cadaveric renal transplants (n=122)	
Kidney	
1 yr	83.6
2 yr	75.9
Patient	
1 yr	92.7
2 yr	89.3
Living-related renal transplants (n=76)	
Kidney	
1 yr	92.1
2 yr	88.0
Patient	
1 yr	98.7
2 yr	94.6
Simultaneous pancreas–kidney transplants (n=100)	
Kidney	
1 yr	91.0
2 yr	85.6
Pancreas	
1 yr	88.0
2 yr	86.8
Patient	
1 yr	94.0
2 yr	92.8

* University of Wisconsin, December 1985–December 1989

Log-rank test: patient survival, p = 0.25; kidney survival, p = 0.12.

patients as potential candidates for SPK transplantation if the theoretical possibility exists that a well-functioning pancreas transplant will stabilize or reverse already existing secondary complications. As shown in Table 1, the average age of our patients was 35 years, with a range of 21 to 53 years. Only 47% of our patients had undergone dialysis before transplantation, and we continue to recommend early transplantation before dialysis. In general we place patients on our waiting list when it seems that end-stage renal failure requiring dialysis will become necessary within 8 to 12 months. Among three patients in our series who had previously undergone amputations, one was due to trauma, and two other patients had transmetatarsal amputations.

Surgical complications (Table 7) occurred with a higher frequency in the DB group. This was previously published by D'Alessandro et al.,[6] comparing the surgical complications of 17 patients transplanted with the DB technique with our initial group of DS patients. This initial experience was confirmed by the now larger group of patients transplanted with the DS technique. The significant reduction of almost all complications experienced in the DS group also reflect our increasing experience in pancreas transplantation and the addition of technical modifications. Clearly the smaller incidence of wound infections in the DS group is related to the fact that all patients who received a pancreas transplant through a bilateral incision were in the DB group, and that bladder leaks also were more frequent in the DB group. Furthermore bleeding complications were more frequent in the DB group. One clear message from our series is that systemic anticoagulation is unnecessary, because only two vascular thromboses have occurred in the entire series. Although three complications were related to the renal transplant, 13 complications were related to the intra-abdominal nature of the transplant procedure.

Characteristic for all types of bladder drainage is the high incidence of urologic complications. Hematuria requiring cystoscopy occurred in 13 patients. In most instances only clot evacuation was required; in fewer instances, however, a distinct bleeding point could be identified and was cauterized. Urethritis causing significant discomfort, mainly a burning sensation during urination, was only seen in men. Therapy consisted of placement of a Foley catheter for 10 to 14 days. Five of our patients required enteric diversion for urethral disruption or hematuria, and all five responded well to conversion.

Infectious complications after SPK transplantation remain frequent and are typically urinary tract infections. Some of these urinary tract infections were caused by the protrusion of nonabsorbable suture material from the pancreaticoduodenal anastomosis. On a few occasions, stone formation around the sutures was noted, and once the stones and sutures were removed cystoscopically, no further urinary tract infections occurred. Because we have changed to the use of absorbable sutures, the problem of bladder stones has been eliminated.

An interesting aspect of this study was a comparison of two groups of patients, one receiving OKT3 induction and the other receiving ALG induction therapy. Although graft and patient survival between these two groups is not different, the higher number of opportunistic infections after OKT3 therapy was remarkable. In particular, CMV infections occurred with significantly higher frequency during the first 6 months after transplantation in the OKT3 group. This increased incidence of opportunistic infections could not be attributed to the total number of rejections requiring therapy, because they were identical in both groups.

Identification of microorganisms recovered from the duodenum before transplantation and correlation with subsequent infections did demonstrate that the bacteriology of the duodenum has no influence on the development of local or urinary tract infections. This could mean that decontamination of the duodenum during the donor procedure is not necessary, or that a quantitatively sufficient reduction was achieved that is sufficient to prevent local infections. Because we cannot differentiate between these two possibilities, we continue our present policy of decontaminating the stomach and duodenum during the donor procedure with povidone iodine and amphotericin.

Despite the high-risk nature of the surgical procedure and the higher incidence of rejections after SPK transplantation, the results 1 and 2 years after transplantation as far as patient and graft survival are concerned are excellent and are comparable to kidney transplantation alone.[11] With the emergence of recent data suggesting that patients undergoing SPK transplantation do not have recurrent diabetic nephropathy in the transplanted kidney,[12,13] and have improvement or stabilization of diabetic neuropathy[14] and microvascular disease, and enhanced quality of life, we now believe that SPK transplantation has emerged as a successful procedure, which is demonstrated to be effective in influencing secondary diabetic complications. Simultaneous pancreas–kidney transplantation should now be considered an effective and therapeutic procedure and should be removed from the list of experimental procedures.

References

1. Sutherland DER, Moudry-Munns KC. Report on the International Pancreas Transplant Registry. Presented at the meeting of the American Gastroenterology Society, May 1990, San Antonio, Texas.
2. Orie JE, Jabi H, Besozzi M, et al. Thallium-201 myocardial perfusion imaging and coronary arteriography in asymptomatic patients with end stage renal disease secondary to juvenile onset diabetes mellitus. Transplant Proc 1986; 18:1709–1710.

3. D'Alessandro AM, Sollinger HW, Hoffmann RM, et al. Experience with Belzer UW cold storage solution in simultaneous pancreas-kidney transplantation. Transplant Proc 1990; 22:532–534.
4. Sollinger HW, Vernon WB, D'Alessandro AM, et al. Combined liver and pancreas procurement with Belzer-UW solution. Surgery 1989; 106:685–691.
5. Sollinger HW, Glass NR, Belzer FO. Clinical experience with pancreaticocystostomy for exocrine pancreatic drainage in pancreas transplantation. Diabetic Nephropathy 1983; 2:23.
6. D'Alessandro AM, Sollinger HW, Stratta RJ, et al. Comparison between duodenal button and duodenal segment in pancreas transplantation. Transplantation 1989; 47:120–122.
7. Nghiem DD, Bentel WD. Duodenocystostomy for exocrine drainage in total pancreatic transplantation. Transplant Proc 1986; 18: 1753.
8. Pirsch JD, Maki DG. Infectious complications in adults with bone marrow transplantation and T-cell depletion of donor marrow: increased susceptibility to fungal infections. Ann Intern Med 1986; 104:619–631.
9. Knechtle SJ, Pirsch JD, Groshek M, et al. OKT3 vs. ALG induction therapy in combined pancreas-kidney transplantation. Transplant Proc 1991; 23:1581–1582.

9a. Wahlberg JA, Love R, Landegaard L, Southard JH, Belzer FO. 72-hour preservation of the canine pancreas. Transplantation 1987; 43:1–4.

10. Sollinger HW, Pirsch JD, D'Alessandro AM, et al. Advantages of bladder drainage in pancreas transplantation: a personal view. Clinical Transplantation 1990; 4:32–36.
11. Sollinger HW, Knechtle SJ: The current status of combined kidney-pancreas transplantation. *In* Sabiston DC, ed. Textbook of Surgery, 14th edition. Philadelphia: WB Saunders, 1990, pp 83–96.
12. Bilous RW, Mauer SM, Sutherland DER, et al. The effects of pancreas transplantation on the glomerular structure of renal allografts in patients with insulin dependent diabetes. N Engl J Med 1989; 321:80–85.
13. Bohman SO, Wilczek H, Tyden G, et al. Recurrent diabetic nephropathy in renal allografts placed in diabetic patients and protective effect of simultaneous pancreatic transplantation. Transplant Proc 1987; 19:2290–2293.
14. Van der Vliet JA, Navarro X, Kennedy WR, et al. The effect of pancreas transplantation on diabetic polyneuropathy. Transplantation 1988; 45:368.

Depressed Gut Absorptive Capacity Early After Trauma-Hemorrhagic Shock

Restoration with Diltiazem Treatment

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Although bacterial translocation occurs after trauma-hemorrhage, it is unknown whether gut absorptive capacity (GAC) is altered under those conditions. The aim of this study, therefore, was to determine this and also whether diltiazem (DZ) has any effect on GAC after hemorrhage. Rats (n = 12; 270 to 300 g) were lightly anesthetized with ether and a 6-cm midline laparotomy performed (*i.e.*, trauma induced). A nasogastric tube was inserted, cannulation of a carotid and femoral artery and a jugular and the portal veins performed, and the animals allowed to recover from anesthesia. They were then bled to a mean blood pressure of 40 mm Hg within 10 minutes and maintained at this pressure by further bleeding or return of fluid (lactated Ringer's solution [LRS]) until 40% of the shed blood volume (SBV) had been returned in the form of LRS (end of hemorrhage, EH; time from onset of hemorrhage to EH = 93.4 ± 4.4 minutes). The rats then were resuscitated with LRS, 3 times the SBV over 45 minutes, followed by 2× over 60 minutes. One group received 400 μg/kg DZ, and another group received saline, with the 2× LRS. Sham animals were not hemorrhaged. Gut absorptive capacity was determined by the 1-hour D-xylose absorption test at 2 and 4 hours after EH. Results demonstrate that GAC is significantly depressed after hemorrhage and resuscitation. Administration of diltiazem restored GAC to normal levels. Thus DZ is a useful adjuvant to treatment after trauma-hemorrhagic shock, because it restores GAC to normal and allows for early enteral nutrition.

THE ROLE OF the gut has been described as the "motor" in the multiple organ failure syndrome in critically ill patients.[1] The importance of bacterial or endotoxin invasion of the bloodstream from the gut causing irreversible shock after hemorrhage was reported 30 years ago.[2] This was largely ignored, however, until it became apparent that many of the patients who suffered multiple organ failure after trauma did so in association with a septic state.[3] Rush et al,[4] Deitch et al,[5] Border et al[6] and Berg[7] have now clearly demonstrated translocation of bacteria from the gut in a number of pathologic states.

Studies also have shown that gut permeability increases,[8] and histologic changes (such as mucosal erosions and submucosal edema) take place in the intestinal mucosa[9,10] after major injury. Despite this, Alexander et al[11] showed conclusively in a prospective randomized study that enteral feeding improved outcome compared with parenteral feeding in burned children. It also has been demonstrated that animals have improved survival after hemorrhage[12] and sepsis[13] if they are enterally, rather than parenterally, fed. Indeed enteral rather than parenteral nutrition has been suggested in trauma patients.[6] From a gut absorptive capacity point of view, however, it has been thought that the gut is quiescent after major injury and sepsis. Ileus is present, nasogastric decompression is necessary, and the patient is managed expectantly until bowel sounds return and flatus is passed.

It has been suggested that the reperfusion of organs after hypoxia induced by trauma-hemorrhage results in a significant increase in cellular calcium levels, which produces deleterious effects on cell and organ function.[14-16] This redistribution of cellular calcium that occurs after low-flow conditions appears to be one of the critical mediators in the pathophysiology of cell death after severe hemorrhage. To prevent altered calcium homeostasis and to maintain cell and organ function after adverse circulatory conditions, a number of investigators have used various calcium channel blockers.[14-16]

The aim of the present study was, therefore, to investigate (1) quantitatively whether gut absorptive capacity is altered after hemorrhage and resuscitation, and (2) the effects of diltiazem on gut absorptive capacity under these conditions.

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Methods

Preparation of Venous Catheters

A piece of Silastic tubing (inner diameter [ID] 0.012 inches, outer diameter [OD] 0.025 inches; Dow Corning, Midland, MI) was inserted into the end of slightly dilated polyethylene (PE) 50 tubing (ID 0.023 inches, OD 0.038 inches; Clay Adams, Parsippany, NJ) and secured with Superglue. In the case of the portal vein catheter, the Silastic portion was 3.5 cm long and the PE portion was 55 cm long. In the case of the jugular vein catheter, the Silastic portion was 2.5 cm long and the PE portion was 45 cm long.

Preparation of Animals

Male Sprague-Dawley rats (275 to 300 g; Sasco, Omaha, NE) were fasted overnight but were allowed free access to water. They were lightly anesthetized with ether, and a 6-cm midline laparotomy was performed using an electric cautery (the ether cone was removed briefly during this procedure).

The ileocecal vein was isolated and cannulated with the Silastic portion of the portal catheter (such that the tip was between the bifurcation of the portal vein and the junction with the splenic vein), and the PE portion of the catheter was exteriorized subcutaneously to the left groin and further tunneled subcutaneously to the dorsal cervical region. The abdomen then was closed in two layers with 3–0 nylon.

A left groin incision was made and the femoral artery isolated. It was cannulated with Silastic tubing (ID 0.020 inches, OD 0.037 inches; Dow Corning, Midland, MI) such that a length of 2.5 cm was inside the vessel. This catheter also was tunneled subcutaneously to the dorsal region of the neck, and the groin incision was closed with 3–0 nylon.

A midline incision was made on the anterior aspect of the neck and the left jugular vein isolated. It was cannulated with the Silastic portion of the jugular catheter, and the PE portion of the catheter was tunneled subcutaneously to the dorsal region of the neck.

The left carotid artery was isolated and cannulated with Silastic tubing (ID 0.020 inches, OD 0.037 inches; Dow Corning, Midland, MI) such that 2.5 cm of the catheter was inside the vessel. The catheter was tunneled subcutaneously to the dorsal cervical region. If the animal was not to be hemorrhaged, the left carotid artery was ligated with 6–0 silk and not cannulated.

Because the animals were not heparinized, slight positive pressure was applied to the venous catheters by infusing normal saline through them at the rate of 300 μL/hr to keep them patent.

A nasogastric tube was inserted after the vascular catheterizations were completed. Silastic tubing (ID 0.025 inches, OD 0.047 inches; Dow Corning, Midland, MI) was inserted through the left nostril into the stomach and secured in position with 6–0 nylon ties.

Hemorrhage Protocol

The unrestrained animal was allowed to awaken and the carotid catheter was attached to a saline manometer for blood pressure monitoring. The rat was bled via the femoral catheter to a mean arterial pressure (MAP) of 40 mm Hg over 10 minutes, and this pressure was maintained by further incremental withdrawal of blood until it was unable to compensate (maximal bleedout [MB]; at this point the animal had shed approximately 60% of its circulating blood volume). An MAP of 40 mm Hg was further maintained by incremental infusion of lactated Ringer's solution (LRS) until 40% of the maximal bleedout volume (MBV) had been returned in the form of LRS (end of hemorrhage [EH]). The animal then was resuscitated by infusing LRS, 3 times the MBV over 45 minutes and a further 2 times over 60 minutes. This was the end of resuscitation (ER).

The animals were divided into the following groups: group 1 was sham, in other words, these animals had all the surgery but were not hemorrhaged or resuscitated; group 2 was hemorrhaged and resuscitated with Ringer's lactate and had 1 mL saline added to the 2× LRS during the resuscitation; group 3 was hemorrhaged and resuscitated with Ringer's lactate and had diltiazem, 400 μg/kg body weight in a total volume of 1 mL saline, added to the 2× LRS. There were four animals in each group.

Parameters Measured

Portal pressure and central venous pressure was measured at the onset of hemorrhage and also at maximal bleedout, end of hemorrhage, end of resuscitation, and just before the D-Xylose absorption test.

Mean arterial pressure was recorded throughout the hemorrhage and resuscitation procedure and also just before the D-Xylose absorption test. Packed cell volume (PVC) also was determined before hemorrhage, and at end of hemorrhage.

Measurement of Gut Absorptive Capacity

Gut absorptive capacity (GAC) was determined using the 1-hour D-Xylose absorption test[17] at 2 hours and 4 hours after the end of hemorrhage. Rats were given a 5% solution (1 mL per 100 g body weight) of D-Xylose (0.5 g/kg body weight)[18] via the nasogastric tube, and 1 hour later 0.3 mL blood was drawn from the portal vein. To make up for fluid loss, the animals were given 0.6 mL LRS for each 0.3 mL blood withdrawn for GAC determinations. The serum was separated off and stored in a freezer until the assay for D-Xylose concentration was to be performed.

D-Xylose Assay

The D-Xylose concentration in blood was measured as described by Allport and Keyser[19] with slight modifications. Briefly, serum was mixed with an equal volume of 20% wt/vol trichloroacetic acid and allowed to stand in ice for 1 hour and then centrifuged. To the supernatant, orcinol-ferric chloride reagent was added, and the mixture was vortexed and heated for 20 minutes in a boiling waterbath, cooled, centrifuged, and the absorbance of the supernatant was measured spectrophotometrically at 660 μm in triplicate against a standard curve.

Statistical Analyses

The results are expressed as mean ± standard error of the mean (SEM). Statistical analyses of the results was performed using the analysis of variance (ANOVA), Tukey's test, and the Newman-Keul's test.

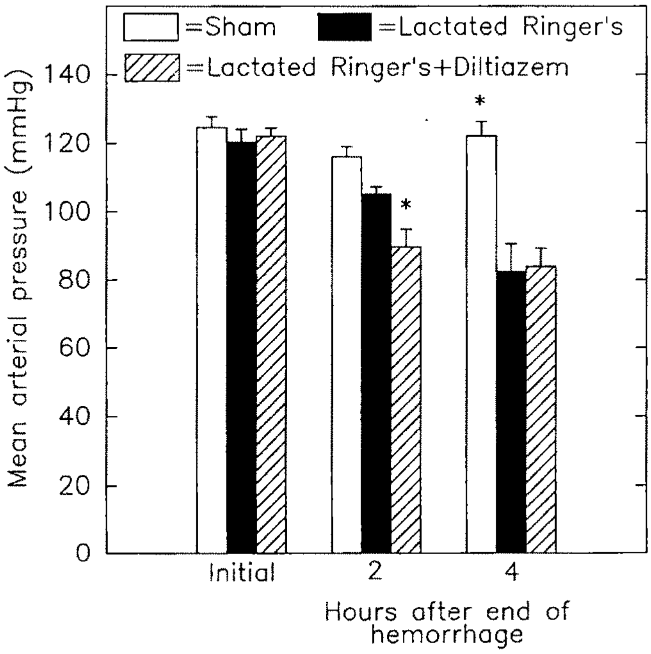

FIG. 1. Effect of hemorrhage and resuscitation on mean arterial pressure (MAP). The animals underwent cannulation of various blood vessels and were hemorrhaged and resuscitated (see Materials and Methods for details). After resuscitation, the MAP was significantly lower in the animals that received diltiazem compared with those that received saline with the lactated Ringer's solution or the sham group at 2 hours after the end of hemorrhage. At 4 hours after the end of hemorrhage, both the hemorrhaged and resuscitated groups of animals had MAP lower than that of the sham group. * = $p < 0.05$, ANOVA, Newman-Keul's test.

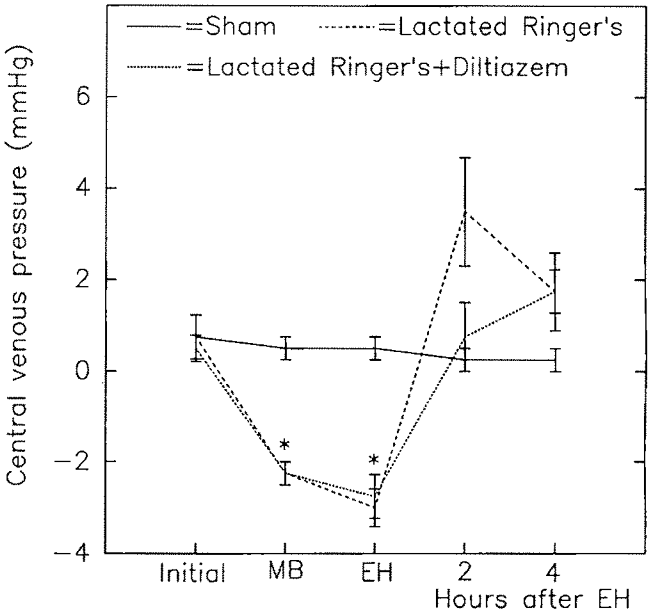

FIG. 2. Effect of hemorrhage and resuscitation on central venous pressure (CVP). The animals underwent cannulation of various blood vessels and were hemorrhaged and resuscitated (see Materials and Methods for details). The CVP was significantly lower than that in sham animals (not hemorrhaged) in groups of animals that were hemorrhaged at maximal bleedout and at the end of hemorrhage. Resuscitation brought the CVP back to normal values, but there was no difference between the animals that received saline or diltiazem with lactated Ringer's solution during resuscitation. MB, maximal bleedout; EH, end of hemorrhage. * = $p < 0.05$, ANOVA, Tukey's test.

Results

Effect of Hemorrhage and Resuscitation on Mean Arterial Pressure

During the hemorrhage procedure, which was 93.4 ± 4.4 minutes from start of hemorrhage to the end of hemorrhage, the MAP of all animals hemorrhaged was maintained at 40 ± 2 mm Hg. Two hours after the end of hemorrhage (*i.e.*, 15 minutes after the end of resuscitation), the MAP was significantly lower in the animals receiving diltiazem (89.75 ± 5.55 mm Hg) compared with the sham (116.00 ± 2.94 mm Hg) or the LRS (105 ± 2.16 mm Hg) animals ($p < 0.05$, ANOVA, Newman-Kuel's test; Fig. 1). By 4 hours after the end of hemorrhage, the MAP of the animals resuscitated with LRS and those receiving diltiazem (82.5 ± 8.15 mm Hg and 84.00 ± 5.29 mm Hg, respectively) was significantly lower than that of the sham animals (122 ± 4.14 mm Hg; $p < 0.05$, ANOVA, Newman-Kuel's test; Fig. 1).

Effect of Hemorrhage and Resuscitation on Central Venous Pressure

The central venous pressure (CVP) was significantly lower ($p < 0.05$, ANOVA, Tukey's test) at maximal bleedout in the animals resuscitated with LRS plus saline (−2.25 ± 0.25 mm Hg) and those with LRS plus diltiazem (−2.25 ± 0.25 mm Hg) compared with the sham group (0.5 ± 0.26 mm Hg; Fig. 2). This was also true at end of hemorrhage (Fig. 2), but at all other points no statistically significant differences were observed. There were no differences in the CVP between the animals receiving saline or diltiazem.

Effect of Hemorrhage and Resuscitation on Portal Venous Pressure

The portal venous pressure was significantly ($p < 0.05$, ANOVA, Tukey's test) reduced at maximal bleedout in the groups receiving saline (4.5 ± 0.26 mm Hg) and diltiazem (3.75 ± 0.63 mm Hg) compared with the sham animals (12.25 ± 0.95 mm Hg). Similar results were seen at end of hemorrhage (Fig. 3). At all other times points, no statistical differences were seen in the portal pressure.

Effect of Hemorrhage and Resuscitation on Hematocrit

The hemorrhage/resuscitation procedure reduced the hematocrit to half the sham values (Fig. 4), but there were no differences seen between the animals receiving saline or diltiazem.

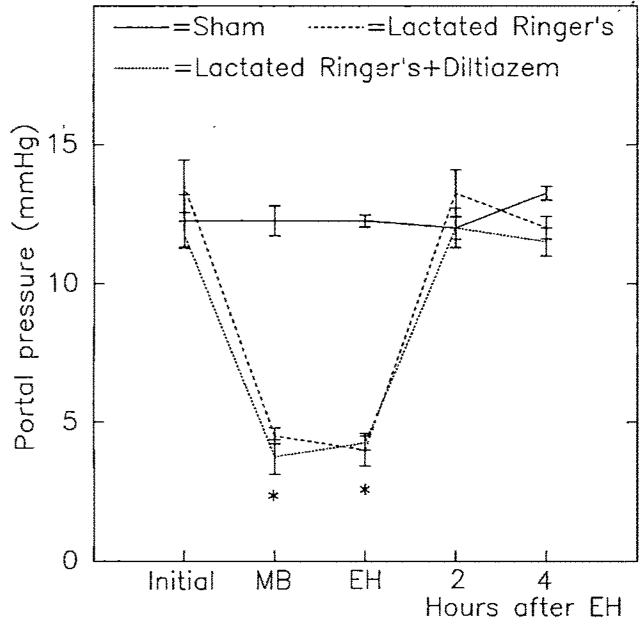

FIG. 3. Effect of hemorrhage and resuscitation on portal venous pressure (PVP). The animals underwent cannulation of various blood vessels and were hemorrhaged and resuscitated (see Materials and Methods for details). The PVP was significantly lower than that in sham animals (not hemorrhaged) in groups of animals that were hemorrhaged at maximal bleedout and at the end of hemorrhage. Resuscitation brought the PVP back to normal values, but there was no difference between the animals that received saline or diltiazem with lactated Ringer's solution during resuscitation. MB, maximal bleedout; EH, end of hemorrhage. * = $p < 0.05$, ANOVA, Tukey's test.

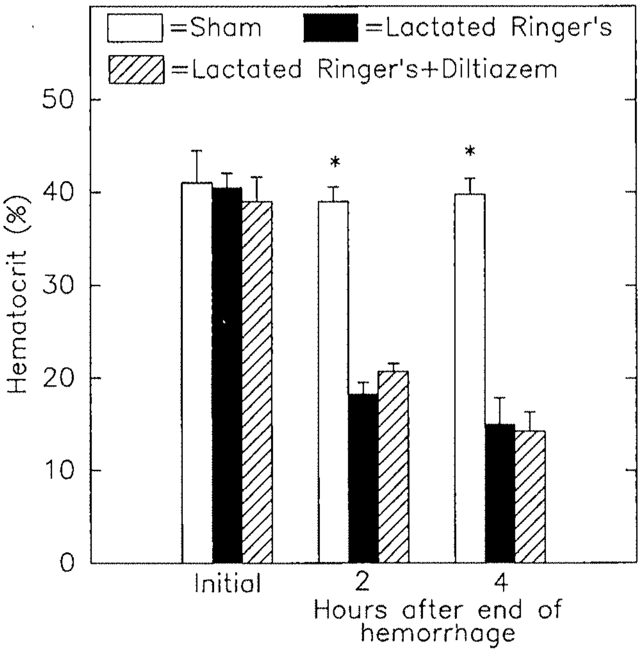

FIG. 4. Effect of hemorrhage and resuscitation on hematocrit value. The animals underwent cannulation of various blood vessels and were hemorrhaged and resuscitated (see Materials and Methods for details). The hematocrit value was significantly lower than that in sham animals (not hemorrhaged) in groups of animals that were hemorrhaged at 2 hours and at 4 hours after the end of hemorrhage. * = $p < 0.05$, ANOVA, Tukey's test.

Effect of Hemorrhage and Resuscitation on D-Xylose Absorption

In the animals receiving saline, D-xylose absorption was significantly ($p < 0.05$, ANOVA, Newman-Kuel's test) reduced at 2 hours after the end of hemorrhage (0.21 ± 0.02 mmol/L) compared with sham animals (0.33 ± 0.01 mmol/L; Fig. 5). This was also the case at 4 hours after end of hemorrhage (0.20 ± 0.02 mmol/L compared with 0.32 ± 0.04 mmol/L; $p < 0.05$, ANOVA, Newman-Kuel's test; Fig. 5).

Diltiazem administration restored D-xylose absorption to normal both at 2 hours (0.40 ± 0.04 mmol/L) and at 4 hours (0.44 ± 0.08 mmol/L) after the end of hemorrhage (Fig. 5).

Discussion

Hemorrhagic shock produces profound α-receptor–mediated vasoconstriction throughout the gastrointestinal tract.[20] The resultant hypoxia is most marked in the mucosal layer, whereas the submucosa and lamina propria appear to be relatively spared. It has been shown that

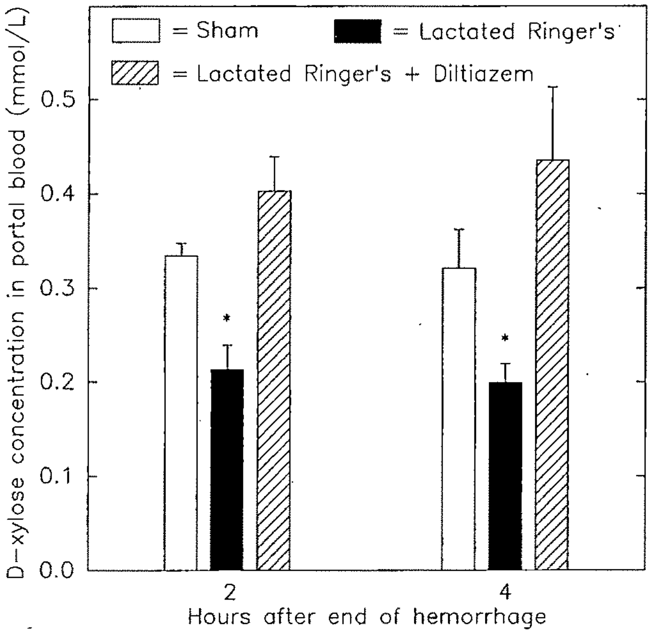

FIG. 5. Effect of hemorrhage and resuscitation on D-xylose absorption. The animals underwent cannulation of various blood vessels and were hemorrhaged and resuscitated (see Materials and Methods for details). The D-xylose absorption was significantly reduced after hemorrhage and resuscitation with lactated Ringer's solution compared with sham animals (not hemorrhaged) at 2 hours and at 4 hours after the end of hemorrhage. The administration of diltiazem 400 μg/kg body weight during the resuscitation restored D-xylose absorption to normal values at both time points. * = $p < 0.05$, ANOVA, Newman-Keul's test.

within 2 hours of severe hemorrhage, there is loss of tight junctions in the mucosal cells of the small intestine,[9] and later there is subepithelial edema[5] and mucosal erosion occurs, starting at the tips of villi.[21] Additionally in the first few days after a severe injury, nutritional management is traditionally neglected because of the need for aggressive fluid resuscitation and other pressing concerns regarding the injuries. Ileus is present and nasogastric decompression is necessary, but that is by and large the extent of the attention paid to the gastrointestinal tract unless there are specific injuries requiring management. As a consequence, the patient loses lean body mass and becomes nutritionally depleted.

Recently attention has been focused on the gut, however, mainly because it is becoming evident as the origin of multiple organ failure in critically ill patients.[1] Furthermore it has been suggested by several investigators, both in the laboratory and in the clinical arena, that early enteral nutrition enhances survival.[6,12,13] It remains unknown, however, whether gut absorptive capacity is affected by severe hemorrhagic shock, and if so, to what degree.

The 1-hour D-xylose absorption test[17] is widely used clinically as a test of malabsorption. Absorption depends on gastric and intestinal motility and the functional integrity of the intestinal mucosa.[22] It is absorbed by the enterocytes of the upper small intestine using the same active mechanism as that for the absorption of glucose[23] and is metabolized in the liver and excreted in the urine.[24] Because of the metabolism of D-xylose by the liver, we decided to measure the concentration of D-xylose in portal venous blood as a more accurate indication of enterocyte activity.

In the model of trauma-hemorrhagic shock used for this study, there is no doubt that a severe hypotensive insult is occurring. The time from onset of hemorrhage to end of hemorrhage was 93.4 ± 4.4 minutes, the CVP was −2.25 ± 0.05 mm Hg, and the portal pressure was reduced by 65% during the period of hypotension. Even using a less severe model of hemorrhage, Kravetz et al.[25] found that the portal pressure was reduced by 27.3% during hemorrhage.

In the model used for this study, we have deliberately not used blood in the resuscitation procedure. The reason for this is that in view of recent concerns about transfusion-related diseases, especially hepatitis and acquired immune deficiency virus (AIDS), we used a model that avoided blood transfusion. There may be concern expressed about erythrocyte replacement because transfusion of crystalloids alone diminishes the oxygen-carrying capacity of blood after significant blood loss. We found that in the hemorrhaged animals, after resuscitation, the hematocrit was reduced to half the initial levels (Fig. 4).

Recent studies in this laboratory have indicated that 50% acute hemodilution with LRS in sham operated animals did not significantly affect cardiac output.[26] Similar observations concerning cardiac output and hemodilution have been reported by others.[27] Furthermore Pelton et al[28] have shown that hematocrits of 10% to 15% in animals subjected to acute hemodilution were well tolerated. Moreover Mesh and Gewertz,[29] in an intestinal ischemia model, have demonstrated that hemodilution has no adverse effects on oxygen consumption during hypotension and hypoperfusion. Indeed they suggest that hemodilution may even be beneficial during reperfusion after ischemia.[29]

Our results indicate that hemorrhagic shock causes a significant decrease in D-xylose absorption despite adequate fluid resuscitation (that this is adequate resuscitation in this model was shown in a previous study from this laboratory[30]) very early on after the injury (Fig. 5). But perhaps more importantly, there is some residual gut absorptive capacity present, and, as we have further demonstrated, the gut absorptive capacity can be improved under such conditions.

The reasons for the diminished gut absorptive capacity may be the actual structural injuries that occur in the small intestine as described above. Because D-xylose is absorbed by the active transport mechanisms used for glucose absorption, however, the problem may lie at a cellular level.

During reperfusion after shock, cellular calcium homeostasis is disrupted, and calcium accumulates with the cell. This accumulation of intracellular calcium may occur either due to a net change in the combined size of the various intracellular cation pools subsequent to alterations in plasma membrane cation permeability, or active cation transport process. Alternatively it may be due to redistribution of the ions within the intracellular compartments after altered intercompartment ion movement by active or passive transport. An uncontrolled rise in cytosolic calcium can initiate a number of intracellular processes, which include excitation–contraction coupling, endocytosis and exocytosis, and enzyme activation. These processes may exacerbate the hemodynamic and metabolic insufficiency underlying ischemic and shock states.[31] Vasoconstriction may produce or worsen ischemia by decreasing nutrient blood flow to cells and tissues. Phospholipase activation by calcium can produce membrane damage and liberate free fatty acids, which stimulate the production of superoxide and hydroxyl radicals and increase the production of eicosanoids. These toxic products may cause further cellular damage. Elevation in cytosolic calcium also may activate proteases, nucleases, calcium-dependent adenosine triphosphatases (ATPases), and may uncouple phosphorylation.[32] Protease activation converts xanthine dehydrogenase to xanthine oxidase. Xanthine oxidase reacts with oxygen and hypoxanthine to produce superoxides and oxygen-free radicals. A number of investigators have used various calcium channel blockers to maintain cell and organ function.[14–16]

We found that diltiazem administration after hemorrhage, in other words, during resuscitation, restored D-xylose absorption to control values (Fig. 5). Diltiazem, a water-soluble calcium channel blocker, has several beneficial effects, including decreased afterload and increased coronary artery blood flow,[33] reduction in catecholamine release,[34] improvement in postischemic microcirculation,[35] sugar transport,[15] decreased lactate production,[36] improved mitochondrial function,[37] and increased tissue ATP.[38] Any or all of these mechanisms may be responsible for the improvement in gut absorptive capacity.

It could be argued that, because D-xylose is using the same transport mechanism as glucose, diltiazem is enhancing that mechanism alone, and that to infer that gut absorptive capacity as a whole is improved by diltiazem is incorrect. Indeed Westfall and Sayeed[15] demonstrated that 3-O-methylglucose transport is enhanced by diltiazem.[15] The dose used was 6 times (2.4 mg/kg) greater than the dose used in this study, however, and it was in isolated skeletal muscle from bacteremic rats. This argument, however, still does not eliminate the possibility that diltiazem is causing a selective improvement in sugar transport, and it would be interesting to see what, if anything, happens to the gut absorptive capacity regarding other nutrients.

It is not known how long gut absorptive capacity remains reduced after severe hemorrhagic shock, and the half-life of diltiazem is 3 to 4 hours. Diltiazem is metabolized in the liver, but hepatic function is also impaired after severe hemorrhagic shock. Thus it remains unknown if further doses of diltiazem would be required to maintain and sustain gut absorptive capacity, and if so, how frequently and for what period.

Calcium channel blockers decrease gastric and intestinal motility, and nifedipine has been suggested as a treatment for the irritable bowel syndrome.[39] This would tend to indicate that gut absorptive capacity would be further diminished because of an additive effect on the ileus already present after severe hemorrhagic shock. Again the doses used to decrease gastrointestinal motility were milligrams per kilogram, rather than the micrograms per kilogram we used in this study.

In summary the results presented in this study demonstrate quantitatively what has been known qualitatively, that gut absorptive capacity is significantly reduced after severe hemorrhagic shock. Nevertheless there is some absorptive capacity still present and diltiazem administration after hemorrhage, in other words, during resuscitation, restores gut absorptive capacity as determined by the one D-xylose absorption test, to control levels. Thus diltiazem appears to be a useful adjunct in the management of patients after severe trauma-hemorrhagic shock, allowing for early enteral nutrition.

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References

1. Carrico CJ, Meakins JL, Marshall JC, et al. Multiple organ failure syndrome. Arch Surg 1986; 121:196–203.
2. Schweinburg FB, Fine J. Evidence for a lethal endotoxemia as the fundamental feature of irreversibility in three types of traumatic shock. J Exp Med 1960; 112:793–800.
3. Polk HC Jr, Shields CL. Remote organ failure: a valid sign of occult intra-abdominal infection. Surgery 1977; 81:310–313.
4. Rush BF, Sori AJ, Murphy TF, et al. Endotoxemia and bacteremia during hemorrhagic shock. The link between trauma and sepsis? Ann Surg 1988; 207:549–554.
5. Deitch EA, Bridges W, Baker J, et al. Hemorrhagic shock-induced bacterial translocation is reduced by xanthine oxidase inhibition or inactivation. Surgery 1988; 104:191–198.
6. Border JR, Hassett J, LaDuca J, et al. The gut origin septic states in blunt multiple trauma (ISS=40) in the ICU. Ann Surg 1987; 206:427–448.
7. Berg RD. Translocation of indigenous bacteria from the intestinal

tract. *In* Human Intestinal Microflora in Healthy and Diseases. Orlando, FL: Academic Press Inc, 1983, pp 333–352.

8. Deitch EA. Intestinal permeability is increased in burn patients shortly after injury. Surgery 1990; 107:411–415.
9. Rhodes RS, Depalma RG, Robinson AV. Intestinal barrier function in hemorrhagic shock. J Surg Res 1973; 14:305–312.
10. Gottlieb JE, Menashe PI, Cruz E. Gastrointestinal complications in critically ill patients: the intensivists' overview. Am J Gastroenterol 1986; 81:227–238.
11. Alexander JW, MacMillan BG, Stinnett JD, et al. Beneficial effects of aggressive protein feeding in severely burned children. Ann Surg 1980; 192:505–517.
12. Zagola GP, Knowles R, Black KW, Prielipp R. Total parenteral nutrition increases mortality after hemorrhage. Crit Care Med 1991; 19:54–59.
13. Kudsk KA, Stone JM, Carpenter G, Sheldon GF. Enteral and parenteral feeding influences mortality after hemoglobin E Coli peritonitis in normal rats. J Trauma 1983; 23:605–609.
14. Gray LS, Gnarra JR, Engelhard VH. Demonstration of a calcium influx in cytolytic T lymphocytes in response to target cell binding. J Immunol 1987; 138:63–69.
15. Westfall MV, Sayeed MM. Effect of diltiazem on skeletal muscle 3-0-methylglucose transport in bacteremic rats. Am J Physiol 1989; 256:R716–R721.
16. Hock CE, Su J, Lefer A. Salutory effects of nitredipine, a new calcium entry blocker, in hemorrhagic shock. Eur J Pharmacol 1984; 97: 37–46.
17. Buts J-P, Morin CL, Roy CC, et al. One-hour blood xylose test: a reliable index of small bowel function. J Pediatr 1978; 90:729–733.
18. Weesner RE, Kolinjivadi J, Giannella RA, et al. Effect of *Strongyloides ratti* on small bowel function in normal and immunosuppressed host rats. Dig Dis Sci 1988; 33:1316–1321.
19. Allport NL, Keyser JW. Pentoses (in blood plasma and tissues). *In* Colorimetric Analysis. Volume 1. London: Chapman and Hall Ltd, 1957, pp 258–260.
20. Marston A. Responses of the splanchnic circulation to ischemia. J Clin Pathol 1977; 30:59–67.
21. Chiu C-J, McArdle AH, Brown R, et al. Intestinal mucosal lesion in low flow states. Arch Surg 1970; 101:478–483.
22. Sammons HG, Morgan DB, Frazer AC, et al. Modification in the xylose absorption test as an index of intestinal function. Gut 1967; 8:348–353.
23. Hindmarsh JT. Xylose absorption and its clinical significance. Clin Biochem 1976; 9:141–143.
24. Wyngaarden JB, Segal S, Foley JB. Physiological disposition and metabolic fate of infused pentoses in man. J Clin Invest 1957; 36:1395–1407.
25. Kravetz D, Sikuler E, Groszmann RJ. Splanchnic and systemic hemodynamics in portal hypertensive rats during hemorrhage and blood volume restitution. Gastroenterology 1986; 90:1232–1240.
26. Wang P, Ba ZF, Chaudry IH. ATP-$MgCl_2$ restores the depressed cardiac output following trauma and severe hemorrhage even in the absence of blood resuscitation. Circ Shock (in press).
27. Heusser F, Fahey JT, Lister G. Effect of hemoglobin concentration on critical cardiac output and oxygen transport. Am J Physiol 1989; 256:H527–H532.
28. Pelton JJ, Cheu HW, McAuley CE. The limit of hemodilutional resuscitation in acute hemorrhagic shock. Surg Forum 1989; 40: 54–57.
29. Mesh CL, Gewertz BL. The effect of hemodilution on blood flow regulation in normal and postischemic intestine. J Surg Res 1990; 48:183–189.
30. Wang P, Chaudry IH. Crystalloid resuscitation restores but does not maintain cardiac output following severe hemorrhage. J Surg Res 1991; 50:163–169.
31. Zaloga GP. Calcium homeostasis in the critically ill patient. Magnesium 1989; 8:190–200.
32. Sayeed MM, Zhu M, Maitra SR. Alterations in cellular calcium and magnesium during circulatory/septic shock. Magnesium 1989; 8:179–189.
33. Nayler WG. Calcium antagonism and the ischemic myocardium. Int J Cardiol 1987; 15:265–267.
34. Nayler WG, Sturrock WJ. An inhibitory effect of verapamil and diltiazem on the release of noradrenaline from ischemic and reperfused hearts. J Mol Cell Cardiol 1983; 16:331–334.
35. Petho A, Neumann T, Vetterlein F, Schmidt G. Influence of diltiazem on postischemic microcirculation and function in the rat kidney. Microvasc Res 1989; 38:223–236.
36. Weishaar R, Ashikawa K, Bing RJ. Effect of diltiazem, a calcium antagonist, on myocardial ischemia. Am J Cardiol 1979; 43:1137–1143.
37. Nagao T, Matlib MA, Franklin D, et al. Effects of diltiazem, a calcium antagonist, on regional myocardial function and mitochondria after brief coronary occlusion. J Mol Cell Cardiol 1980; 12:29–43.
38. Watts J, Maiorano P, Harwell T. Comparison of the effects of bepridil and diltiazem upon globally ischemic rat hearts. Eur J Pharmacol 1987; 134:25–33.
39. Traube M, McCallum RW. Calcium-channel blockers and the gastrointestinal tract. Am J Gastroenterol 1984; 79:892–896.

The Effect of Glucocorticoid Administration on Bacterial Translocation

Evidence for an Acquired Mucosal Immunodeficient State

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Adherence of bacteria to intestinal epithelial cells may be the crucial initiating event for translocation and is normally prevented by both specific (secretory IgA) and nonspecific (mucus, bacterial antagonism, desquamation) mucosal defense mechanisms. The purpose of this study was to examine the effect of dexamethasone administration on mucosal immunity; specifically bacterial adherence and IgA. Twenty Fischer rats were randomly assigned to two groups of 10 animals each. Group I received 0.5 mL saline injection intraperitoneally (IP); and group II, 0.8 mg/150 g body weight dexamethasone IP per day for 2 consecutive days. The cecum, mesenteric lymph nodes, and bile were aseptically collected, and bacterial adherence, bacterial translocation, and IgA concentration were determined. Results indicate that, compared with saline-treated animals, dexamethasone-treated animals had a fall in IgA (54 ± 24 *versus* 232 ± 41 μg/mg protein), an increase in bacterial adherence (8.2 ± 0.5 *versus* 3.4 ± 0.6 cfu (log^{10})/g cecum), and an increased incidence of bacterial translocation to the mesenteric lymph nodes (60% *versus* 0%). These data suggest that glucocorticoids may promote bacterial translocation by impairment of mucosal IgA synthesis.

SECRETORY IMMUNOGLOBULIN A, THE MOST abundant immunoglobulin on mucosal surfaces, is the principle component of the mucosal immune system. Synthesis and expression of s-IgA into secretions prevents the adherence of bacteria and viruses to mucosal epithelial cells, thereby hindering their pathogenicity and shielding the systemic immune system from further activation.[1] Immunoglobulin A (IgA) is synthesized by plasma cells in the gut lamina propria and is expressed onto the mucosal epithelial surface and into the lumen in association with J chain and secretory component. Secretory component acts as a receptor on the basolateral surface of enterocytes, binding to dimeric IgA and facilitating its transport to the lumen.

We have previously reported that total parenteral nutrition promotes bacterial translocation to mesenteric lymph nodes in association with a marked diminution in both biliary secretory IgA as well as marked depletion of gut lamina propria plasma cells of the IgA isotype.[2,3] Bacterial translocation has been reported after several models of catabolic stress, including burns, shock, sepsis, and trauma.[4] Burn models result in a 90% reduction in biliary IgA concentration as early as 18 hours after the burn injury.[5] Glucocorticoids administered at physiologic doses result in significant decreases in IgA and IgG at the mucosal surface.[6] Because endogenous glucocorticoid release can be demonstrated in virtually all animal models involving a severe catabolic stress,[7] it was the purpose of this study to examine the effect of dexamethasone, a pure glucocorticoid, on mucosal IgA, bacterial adherence to the mucosa, and bacterial translocation.

Materials and Methods

Twenty female Fischer rats weighing between 100 and 150 g were randomly assigned to two groups of 10 animals each. Group I (SALINE) received 0.5 mL 0.9% NaCl injection intraperitoneally (IP)/day in two divided doses for 2 consecutive days. Group II (DEX) received 0.8 mg/150 g body weight of dexamethasone IP/day in two divided doses for 2 consecutive days. Animals were allowed *ad libitum* access to water during the study period but were otherwise made *nulla per os* (NPO). Forty-eight hours after the initiation of the study, animals were anesthetized with pentobarbital (50 mg/kg IP) and ketamine (25 mg/kg IP), and sterilely prepared and shaved for abdominal exploration using 30% alcohol. The common bile duct was skeletonized just proximal to the mesoduodenum and cannulated using Silastic tubing as previously described.[8] Bile was collected into a sterile container for 15 minutes

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from five animals in each group. Next all animals underwent an *en bloc* excision of the mesenteric lymph nodes by aseptically dissecting the mesenteric chain of lymph nodes from the ileocecal valve to the root of the mesentery. The cecum was excised and its contents (stool) were removed for culture. The cecum was placed in a sterile petri dish and vigorously washed using a jet stream of 0.9% NaCl until the wash effluent was clear. The washed cecum was submitted for culture.

Mesenteric lymph nodes (MLN) were weighed, transferred to a sterile 2.0 Ten Broek tissue grinder (Corning Glass Works, Corning, NY), and diluted 1:10 (wt/vol) with sterile 0.85 NaCl. After manual grinding, each sample was spread plated on five TSA II blood Agar plates (BBL, Becton Dickson, Co., Cockeyesville, MD). Representative colonies were expressed as colony-forming units per gram of organ tissue (cfu/g) and converted to $\log_{10}$. Tissues were considered to be "positive" for bacteria if greater than 100 cfu/g of tissue was found and at least three of the five plates were growing bacteria.

The cecum and stool of each rat was also weighed, transferred to sterile tissue grinders, and diluted as above. Homogenized samples then were serially diluted 10-fold in sterile 0.85 NaCl. Nine spread plates of each dilution were made on EMB Agar (Difco Labs, Detroit, MI). Representative colonies from the EMB plates were identified by the hospital clinical laboratory using standard techniques. Results were expressed in cfu/g of cecum($\log_{10}$). Colony counts from EMB plates were used to distinguish two groups of gram-negative bacteria, non–lactose- and lactose-fermenting bacteria.

Bile was assayed for IgA by enzyme-linked immunosorbent assay (ELISA) as previously described.[9]

Protein was assayed using the Lowry method (Sigma Chemical Co., St. Louis, MO). Total IgA in bile was expressed as the concentration of IgA per milligram bile protein (μg/mg protein) to normalize for variability in bile flow or liver blood flow between animals.

Cecal stool was diluted and microscopically examined for IgA coating of bacteria using fluorescein isothiocyanate (FITC)-labeled specific antisera using a modification of the method described by Vidhichamnong et al.[10] Briefly, stool was diluted 1:10 (wt/vol) in phosphate-buffered saline (PBS) and the heavy debris was allowed to settle at 30 C. One hundred microliters of the supernatant was removed and placed on a glass slide, and the bacteria was fixed to the slide by heating. The slide then was incubated with several dilutions of FITC-labeled anti-IgA antisera, washed several times with PBS, and then allowed to dry in the refrigerator. Using both phase contrast and fluorescent microscopy, rods were identified and evaluated as to their fluorescence on a scale of 1 to 5. Fluorescence was expressed as intensity units (IU) per high-power field (hpf).

Statistical Analysis

Translocation rates were tested for level of significance using a chi-square analysis with a Yates correction for continuity. Culture results were compared between groups using a Mann-Whitney U test because of the nonparametric nature of these data. Data for IgA were tested for significance using a the Mann-Whitney sum rank analysis.

Results

All animals survived the 2-day study period. Animals that received dexamethasone developed a mild septic appearance manifested by piloerection, conjunctivitis, and a slight degree of hypoactivity.

Animals receiving saline for the 2-day study period appeared healthy without any signs of illness. Dexamethasone-treated animals (Group II) had a statistically significant decrease ($p < 0.01$) in biliary IgA compared with the saline-treated animals (DEX-54 ± 24 *versus* SALINE-232 ± 41 μg/mg protein ± standard error of the mean [SEM]). In addition a statistically significant decrease ($p < 0.01$) in IgA coating of bacteria was observed in the dexamethasone-treated animals compared with the saline group (DEX, 1.4 ± 0.5 *versus* SALINE, 3.8 ± 1.1 IU/hpf ± SD) (Fig. 1). Dexamethasone-treated animals developed a statistically significant increase ($p < 0.05$) in the incidence of bacterial translocation to the mesenteric lymph nodes compared with saline-treated animals (DEX, 60% *versus* SALINE, 0%) (Fig. 2). The most common bacteria cultured in the MLNs was *Escherichia coli*, followed by *Proteus vulgaris*. This increased translocation rate was accompanied by a statistically significant increase in both the gram-negative bacterial concentration in stool (STOOL) and the gram-negative bacterial concentration adherent to the cecal wall (CECUM); (STOOL: DEX, 9.2 ± 0.4 *versus* SALINE, 7.7 ± 0.9 cfu ($\log_{10}$)/g stool ± stan-

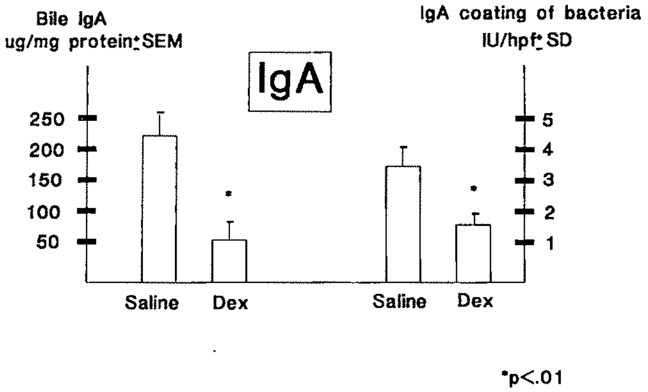

FIG. 1. IgA concentration in bile and IgA coating of bacteria. A statistically significant decrease ($p < 0.01$, Mann-Whitney) was achieved between biliary concentration of IgA and IgA coating of bacteria in the dexamethasone-treated animals.

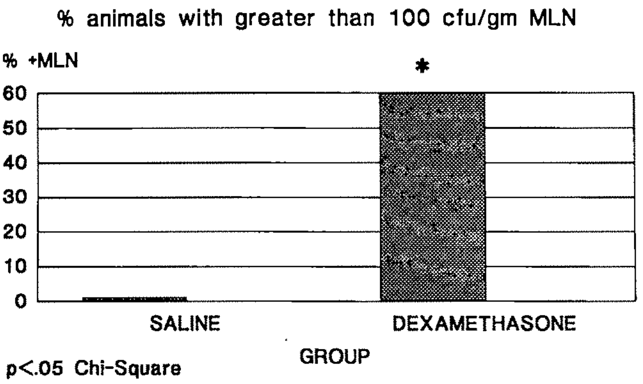

FIG. 2. Incidence of bacterial translocation to mesenteric lymph nodes (MLN) between groups. A statistically significant difference ($p < 0.05$ chi square) between translocation rates can be seen.

dard deviation [SD]; CECUM: DEX, 8.2 ± 0.5 *versus* SALINE, 3.4 ± 0.6 cfu $(\log_{10})$/g cecum ± SD) (Fig. 3).

Discussion

Bacterial translocation from the gut, the extraintestinal relocation of the indigenous intestinal microflora, can occur to a significant degree after a variety of stresses, which include burns, trauma, shock, anaerobic decontamination, parenteral nutrition, radiation, and chemotherapy.[11] Two basic findings common among these animal models appear to be alteration of the normal intestinal microbial balance and physical or immunologic disruption of the normal epithelial barrier to bacteria.[12]

Normal enteric bacteria are confined to the intestinal lumen by a complex interplay of luminal, mucosal, cellular, and immunologic/inflammatory mechanisms. By this reasoning interference of bacterial migration can occur intraluminally, at the mucosal surface, or in passage by a complex repertoire of bactericidal activity present in all layers of the intestines. Intestinal immune function therefore is a general term describing the prevention of passage of viable bacteria, viruses, or toxins from the gut lumen to any extraintestinal site and may involve immunoglobulins, oxygen radicals, macrophages, etc. Mucosal immune function, however, describes an earlier step in the translocation process and is defined as prevention of invasion of the surface epithelial cell by inhibition of adherence of luminal pathogens to the cell surface. Adherence of bacteria to the intestinal epithelial cell is thought to be the crucial initiating event for the establishment of mucosal invasion.[13] A vast array of well-described receptors for bacterial adhesins exists on mammalian cells, and interference with the adherence factors on pathogenic bacteria prevents their ability to cause mucosal disease (Table 1).[14] For example when adherence of diarrheagenic enteroinvasive bacteria has been prevented, they can no longer cause diarrhea even when introduced into the gastrointestinal tract in high concentration.[15] Bacteria have specialized pili or fimbriae that serve as the adhesin or virulence factor, permitting attachment to epithelial cell receptors. Bacterial adherence is normally prevented by both specific (secretory IgA) and nonspecific (mucus, bacterial antagonism, desquamation) mucosal defense mechanism.[16] Normally the anaerobic bacteria form a paste on the mucosal surface. Their presence has been shown to sterically inhibit the epithelial cell receptors for adhesins on gram-negative bacteria.[17] Anaerobic bacteria as a rule do not translocate across histologically normal intestine; however decontamination of the anaerobic flora with antibiotics results in translocation of gram-negative organisms.[18] Mucus is also an important physical barrier for bacteria, and its absence results in significant bacterial adherence.[19] If bacteria are able to breach these defense barriers, cell desquamation can help debride the bacteria. This apparently occurs only when the cell perceives a critical number of surface-colonizing bacteria.[20] Although these nonimmune mechanisms are important for mucosal defense, the only immune-specific mecha-

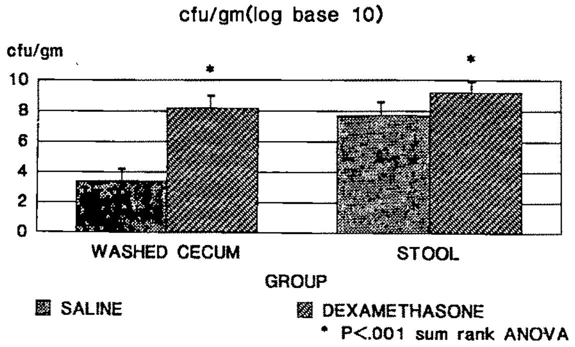

FIG. 3. Cecal and stool culture results. Stool and ceca were homogenized and plated on EMB agar, which is selective for gram-negative bacteria. A statistically significant increase ($p < 0.001$, Mann-Whitney) was observed in both cecal and stool bacterial concentrations in the dexamethasone-treated animals. This increase appeared particularly striking in the washed cecum.

TABLE 1. *Examples of Adhesin/Receptor Interaction**

Microorganism	Adhesin	Receptor
Esherichia coli	Type 1 fimbriae	D-mannose
	p-fimbriae	
	K88 fimbriae	alpha-D-Galp(1-4)-B-Galp
	K99 fimbriae	B-D-Gal(GM ganglioside)
	CFA 1 fimbriae	GM-2 ganglioside
		GM-2 ganglioside
Pseudomonas aeruginosa	Fimbriae	Sialic acid residue
Staphylococcus aureus	Lipoteichoic acid	Fibronectin

* To establish invasion, bacterial adhesions attach to epithelial cell receptors in lock-and-key fashion. These adhesion/receptor interactions account for the preponderance of potentially pathogenic resident flora to invade at specific sites.

nism for the prevention of bacterial adherence is the coating of bacteria by secretory IgA.

Secretory IgA (s-IgA) is a 360,000 MW immunoglobulin synthesized by gut lamina propria plasma cell originating as Peyer's patch B-cells.[1] Antigen-specific s-IgA is released into secretions after antigenic challenge in Peyer's patches and homing of B-cells to remote sites such as breast, salivary gland, intestine, and liver.

S-IgA coats bacteria and prevents bacterial adherence by an unknown mechanism. S-IgA is resistant to proteolysis, contains J-chain and secretory pieces, and is held together by several disulfide bridges, permitting its survival at varying temperatures and pHs, such as are encountered in the gastrointestinal lumen. This antigen-specific immunoglobulin appears to have a particular affinity for the gram-negative bacteria that posses the necessary adhesins for attachment to human and animal gut mucosa. IgA coating of bacteria has been demonstrated to be selective for the gram-negative enteric flora in humans and rodents.[21] A careful study by Van der Waaj and Berghuis[22] demonstrated that approximately 60% to 80% of the gram-negative enteric organisms in the human and rodent gastrointestinal tract are IgA coated, whereas only 10% to 20% of gram-positive organisms were coated.[22]

Data from the present study indicate that the most common translocating organism was *E. coli.* In addition cecal homogenates grown on media selective for gram-negative bacteria demonstrate that significant adherence of gram-negative bacteria occurred. It is interesting that in virtually all the human and animal reports where bacterial translocation has been demonstrated, the most common organisms isolated are the *enterobacteriaceae.*[23] Furthermore both IgA concentration in fluids and IgA coating of bacteria has been demonstrated to be decreased in many of these models, such as parenteral nutrition, radiation, and chemotherapy.[22] It is attractive to speculate that IgA deficiency may lead to adherence of gram-negative bacteria to the mucosal epithelial cell and that this is the crucial initiating event for bacterial translocation. The causal relationship between bacterial translocation and IgA, however, remains to be proven.

Data from the present study demonstrate that dexamethasone administration results in a significant decrease in biliary IgA and IgA coating of cecal bacteria associated with bacterial adherence to the cecum and bacterial translocation to mesenteric lymph nodes. Wira has recently reported that dexamethasone administration, in physiologic doses, results in a marked decrease in IgA in saliva and vaginal secretions of rats.[6] In addition antigen-specific IgA production after oral antigenic challenge was significantly attenuated in dexamethasone-treated animals.[6] These findings were associated with a significant increase in serum polymeric IgA. These investigators suggested that endogenous glucocorticoids might enlist antibodies at the mucosal surface to confer immune protection systemically. Data from the Wira study and data from the present study beg the question: Is the fall in mucosal IgA from stress-induced glucocorticoid release beneficial or harmful to the host? Data from the present study suggest that glucocorticoids may result in harm to the host, especially because the animals in the dexamethasone group developed chromodacryorrhea. Bacterial translocation to mesenteric lymph nodes may be a sample process whereby the host is able to survey enteric pathogens with potential to disseminate. In this manner induction of a backup serum immune response such as a rise in polymeric IgA, as Wira would suggest, occurs. In the process of bacterial translocation to the mesenteric lymph nodes, however, release of macrophage-derived cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1) can be released and result in a severe systemic inflammatory response.[24] To what extent the translocation of bacteria is helpful or harmful to the host is therefore not yet clear. Just as low levels of TNF may be important for the induction of the inflammatory response to invading pathogens, low levels of bacteria in the mesenteric lymph nodes may be appropriate during the initial phase of the catabolic response to sepsis. Persistence or dissemination of bacteria within the mesenteric lymph nodes, however, may be harmful.

Because bacterial translocation can be demonstrated in such a wide variety of animal models of stress, data from the present study suggest that glucocorticoid release may be a common stimulus in these models. Furthermore because a significant diminution in IgA can be demonstrated in many models of stress, such as burn injury, the mechanism of glucocorticoid-induced bacterial translocation may be by impairment of IgA synthesis and function.[7] Data from Figure 3 demonstrate that differences in bacterial adherence between the groups was much more dramatic than the differences in stool bacterial counts. A near five order of magnitude difference in the $\log_{10}$ cfu/g of bacterial adherence to the cecal wall is indicative of the substantial impairment in "mucosal" immunity observed in the dexamethasone-treated animals. Although IgA does not represent the only mucosal defense mechanisms that prevents adherence of bacteria, the 76% reduction in biliary IgA observed in the dexamethasone-treated animals is likely to contribute significantly to the mucosal immune impairment. This is especially true in view of the fact that 90% of intestinal IgA in rodents, in contrast to humans, is derived from bile.[25] These data are further substantiated by the significant reduction in IgA coating of bacteria in the dexamethasone-treated animals.

In summary dexamethasone-treated animals develop bacterial translocation to the mesenteric lymph nodes in association with a marked decrease in IgA concentration in bile. Whether patients with multiple organ failure states die *of* or *with* bacterial translocation remains an important focus of future studies in humans.

References

1. McNabb PC, Tomasi TB. Host defense mechanisms at the mucosal surface. Annu Rev Microbiol 1981; 138:976–983.
2. Alverdy JC, Aoys E, Moss GS. Total parenteral nutrition promotes bacterial translocation from the gut. Surgery 1988; 104:185–190.
3. Alverdy JC, Weiss-Carrington P, Aoys E, et al. The effect of total parenteral nutrition on gut lamina propria plasma cells. JPEN 1990; 14(suppl):8.
4. Rush BF, Sori AJ, Murphy TF, et al. Endotoxemia and bacteremia during hemmorrhagic shock: the link between trauma and sepsis. Ann Surg 1988; 207:549–554.
5. Harmatz PR, Carter EA, Sullivan D, et al. Effect of thermal injury in the rat on transfer of IgA protein into bile. Ann Surg 1989; 210:203–207.
6. Wira CR, Sandoe CP, Steele MG. Glucocortoid regulation of the humoral immune system. J Immunol 1990; 144:142–146.
7. Moyer E, Cerra F, Chenier R, et al. Multiple systems organ Failure. Death predictors in the trauma-septic state—the most critical determinants. J Trauma 1981; 21:862–869.
8. Alverdy JC, Moss GS, Sheldon GS. A model for chronic intermittent bile sampling in an unrestrained rat. Surg Res Commun 1988; 3:47–52.
9. Burke DJ, Alverdy JC, Aoys E, et al. Glutamine-supplemented total parenteral nutrition improves gut immune function. Arch Surg 1989; 124:2396–2399.
10. Vudhichamnong K, Walker DM, Ryley HC. The effect of secretory immunoglobulin A on the *in-vitro* adherence of the yeast *Candida Albicans* to human oral epithelial cells. Arch Oral Biol 1982; 27: 617–621.
11. Carrico J, Meakins JL. Multiple organ failure syndrome. Arch Surg 1986; 121:196–208.
12. Wells CL, Jechorek RP, Erlandsen SL, et al. The effect of dietary glutamine and dietary RNA on ileal flora, ileal histology, and bacterial translocation in mice. Nutrition 1990; 6:70–75.
13. Ofek I, Beachey EH. Bacterial adherence. Adv Intern Med 1980; 25:503–532.
14. Gaastra W, Degraaf FK. Host-specific fimbrial adhesins of noninvasive enterotoxigenic *Escherichia coli* strains. Microbiol Rev 1982; 46:129–161.
15. Levine MM, Nataro JP, Kaarch H, et al. The diarrheal response of humans to some classic serotypes of enteropathogenic *Escherichia coli* is dependent on a plasmid encoding of an enteroadhesiveness factor. J Infect Dis 1985; 152:550–559.
16. Abraham SN, Beachey EH. Host defenses against adhesion of bacteria to mucosal surfaces. *In* Advances in Host Defense Mechanisms, Vol. 4. New York: Raven Press, 1985, pp 63–88.
17. Bibel JD, Aly R, Bayles RA, et al. Competitive adherence as a mechanism of bacterial interference. Can J Microbiol 1983; 29:700–703.
18. Deitch EA, Maejima K, Berg R. Effect of oral antibiotics and bacterial overgrowth on the translocation of the GI tract microflora in burned rats. J Trauma 1985; 25:385–392.
19. Parsons CI, Mulholland SG, Anwar H, et al. Antibacterial activity of bladder surface mucin duplicated by exogenous glycosaminoglycan(heparin). Infect Immun 1979; 24:555–557.
20. Gibbons RJ, Van Houte J. Bacterial adherence in oral microbiology. Annu Rev Microbiol 1975; 29:19–44.
21. Van Saene DKF, Van Der Waaij D. A novel technique for detecting IgA-coated potentially pathogenic microorganisms in the human intestine. J Immunol Methods 1979; 30:87.
22. Van Der Waaij, Berghuis JM. Determination of the colonization resistance of the digestive tract of individual mice. J Hyg (Cambr) 1973; 72:379.
23. Wells CL, Maddaus MA, Simmons RL. Proposed mechanisms for the translocation of intestinal bacteria. Rev Infect Dis 1988; 10: 958.
24. Wilmore D, Smith R, O'Dwyer S, et al. The gut: a central organ after sepsis. Surgery 1988; 104:917.
25. Lemaitre-Coelho I, Jackson GDF, Vaerman JP. Relevance of biliary IgA antibodies in rat intestinal immunity. Scand J Immunol 1978; 8:459–463.

Surgical Management of Primary Lymphoma of the Breast

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Review of our surgical experience with 10 cases of "primary" lymphoma initially confined to the breast and of previous published reports indicates that radical surgery is to be avoided. Radiation therapy with limited surgery provides excellent local–regional control. The addition of systemic combination chemotherapy is warranted in the more aggressive histologic subtypes because death is generally due to subsequent disseminated disease.

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PRIMARY LYMPHOMA OF the breast constitutes less than 1% of malignant breast neoplasms.[1-21] The frequency with which the various histopathologic types of primary breast lymphoma occur is difficult to determine, because terminology varies among the relatively small cases series that have been reported. There is general agreement, however, that these lymphomas are usually of non-Hodgkin's histology.[13] Because non-Hodgkin's lymphoma is considered a disease of multifocal origin, the appropriate surgical management must take into account the systemic nature of the so-called primary lymphoma of the breast.[10-14] Although there are approximately 250 published cases in the Western literature of primary breast lymphoma,[12] most series do not address the surgeon's role in the treatment of lymphoma that presents as disease initially confined to the breast. We reviewed the surgical experience in 10 cases of primary breast lymphoma that were treated between 1944 and 1990 at The University of Texas M. D. Anderson Cancer Center to determine whether the extent of surgery had an impact on regional disease control and subsequent outcome.

Patients and Methods

The 10 surgical patients were staged at the time of initial diagnosis using the Ann Arbor Staging System[15] for primary extranodal lymphomas: IE, disease localized to a single extranodal area (breast) or IIE, disease localized to an extranodal area (breast) with regional lymph node involvement. The histopathologic diagnoses were classified according to the Working Formulation and Rappaport classification systems.[16,17] Patients with lymphomas at other sites, with axillary nodal involvement only, or with chest wall lymphomas, were excluded. The mean follow-up was 50.8 months, with a range of 5 to 154 months. One patient was lost to follow-up after excisional biopsy only.

Results

Clinical Features

All 10 patients were white women with a mean age of 53 years (range, 17 to 71 years). The initial chief complaint in each case was a rapidly enlarging breast mass of a mean duration of 15.5 weeks (range, 3 weeks to 12 months). "B" symptoms (10% weight loss, night sweats, fever) were reported in only two patients (Table 1).

All patients had a unilateral, well-circumscribed breast mass without chest wall fixation (Fig. 1A). Skin changes with nipple retraction were described in only one patient. The average tumor size was 5.75 cm in diameter, with a range of 2 to 15 cm. Six cases involved the right breast, with the other four occurrences in the left breast. No patient presented with bilateral synchronous breast lymphoma. One patient developed a contralateral breast lymphoma 4 months after initial diagnosis, however. Clinically involved ipsilateral axillary nodes were present in two patients.

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TABLE 1. *Summary of 10 Patients With Primary Lymphoma of the Breast*

Patient	Tumor Size (cm)	Stage	Histologic Findings	Treatment	Disease-free Interval (mo)	Status (last follow-up)
1	0	IEA	Small lymphocytic (well-differentiated lymphocytic)	Excisional Bx, XRT	151	Dead
2	8	IIEA	Follicular:mixed small/large cell (nodular mixed)	Modified mastectomy, XRT	4	Dead
3	2.5	IEA	Diffuse large cell (diffuse histiocytic)	Excisional Bx, chemotherapy	22	NED
4	5.5	IEA	Diffuse large cell (diffuse histiocytic)	Radical mastectomy, XRT, chemotherapy	117	NED
5	7	IEB	Diffuse large cell (diffuse histiocytic)	Modified mastectomy, chemotherapy	97	Dead
6	—	IEB	Diffuse large cell (diffuse histiocytic)	Excisional Bx, chemotherapy, XRT	8	Dead
7	—	IEA	Diffuse large cell (diffuse histiocytic)	Excisional Bx, chemotherapy, XRT	13	NED
8	4	IEA	Diffuse small cell (diffuse poorly differentiated lymphocytic)	Excisional Bx	—	Unknown
9	2	IEA	Diffuse large cell (diffuse histiocytic)	Excisional Bx, chemotherapy, XRT	7	NED
10	15	IIEA	Diffuse large cell (diffuse histiocytic)	Needle Bx, chemotherapy, XRT	5	Distant metastasis

Bx, biopsy; XRT, radiation therapy; NED, no evidence of disease.

Mammograms were available for six patients and usually showed a homogeneous, fairly well-demarcated mass density without tumor spiculations or malignant-appearing calcifications (Fig. 1B).

Histologic Classification

Eight cases were classified as diffuse lymphomas, of which seven were large-cell type (diffuse histiocytic) and one was small-cell (diffuse poorly differentiated lymphocytic) (Table 1). The other two cases were classified as a mixed small- and large-cell (nodular mixed) and a small-cell lymphocytic (well-differentiated) lymphoma.

Treatment

Surgical treatment included excisional biopsies only in six patients, modified radical mastectomy in two, and a radical mastectomy in one.

Radiation therapy was given to four patients after excisional biopsy, to two patients after mastectomy, and to one patient who had needle biopsy only. The total radiation dose was usually 40 Gy (range, 38 to 45 Gy).

Systemic chemotherapy was given to seven patients: two who had surgery alone, four who had surgery and radiation therapy, and one who had needle biopsy diagnosis and radiation therapy. The chemotherapy regimen usually included cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin, given every 3 weeks for 6 to 24 cycles.

Survival

Four patients (cases 1, 2, 5, and 6) died from disseminated lymphoma; overall survival durations were 154, 6, 102, and 32 months (disease-free survivals were 151, 4, 97, and 8 months), respectively (Table 1). The five survivors (cases 3, 4, 7, 9, and 10) have had disease-free survivals of 22, 117, 13, 7, and 5 months to the date of last follow-up. None of the 10 patients developed recurrence of the ipsilateral breast or chest wall regardless of surgical therapy.

Discussion

The clinical presentation of our patients with breast lymphoma is similar to that reported by others. The average age is the fifth decade of life,[13,14] which is also the mean age of women with adenocarcinoma of the breast.[18] The history is usually one of a rapidly expanding, painless mass; there is a slight predilection for the right breast.[3,5,12–14] Although only one patient developed bilat-

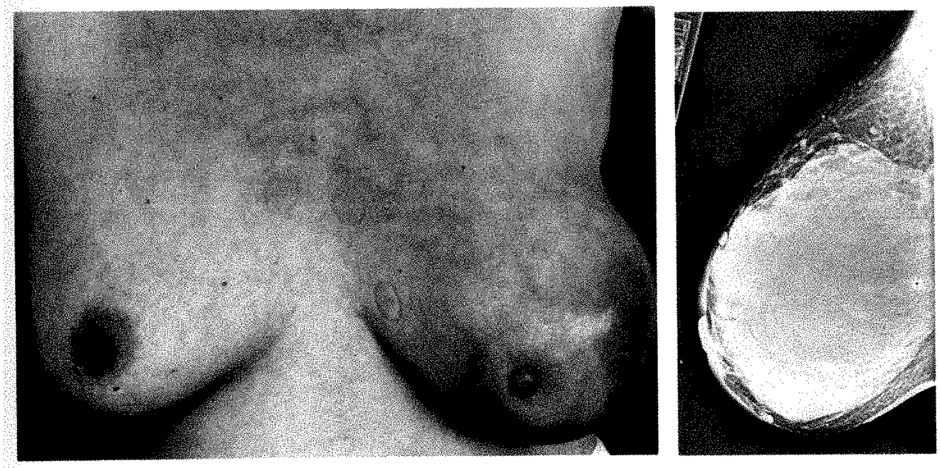

FIG. 1. A, A 58-year-old woman had a rapidly enlarging, painless mass in the left breast. B, Mammogram showed a well-defined mass that lacked tumor calcifications or significant surrounding distortion that is often associated with primary breast carcinoma. After two cycles of systemic chemotherapy followed by radiation therapy, the patient had minimal residual tumor (<1 cm) in the breast, but a subcutaneous distant metastasis developed in the lower back.

eral lymphomas of the breasts, an incidence of 6% to 13% has been reported.[6,14,19,20] This characteristic clinical history and mammographic finding of a diffuse homogeneous mass should alert the surgeon to consider lymphoma in the differential diagnosis.[21]

As in other series,[13,14] the most frequent histology we observed was diffuse large-cell (histiocytic) lymphoma. Although this histology is usually associated with an aggressive course,[14] two of our patients survived 117 and 102 months. This survival may reflect the primary tumor's sensitivity to chemotherapy and the concomitant control of occult systemic disease. Recent data indicate that second and third generations of chemotherapy combinations derived from the original CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) protocol have complete response rates as high as 70% to 80%.[22] The effectiveness of these new chemotherapy regimens and the high propensity of poor prognostic histologic types to disseminate rapidly support the evolving concept that aggressive treatment with systemic therapy is the optimal approach and that radical surgery should be avoided.[9,10,14,23] In patients with localized well-differentiated lymphomas (case 1), conservative surgery and radiotherapy may achieve excellent results.

Thus the surgeon must not only recognize the clinical entity of lymphoma of the breast, but also must understand its biology and the need for multimodality therapy. After obtaining histologic diagnosis by biopsy, the extent of disease should be staged by chest radiographs, bone marrow biopsy, computed tomography (CT) scans of the abdomen, and possibly lymphangiogram. Depending on stage and histologic classification, radiation therapy can be used for local control with systemic chemotherapy.

References

1. Adair FE, Hermann JB. Primary lymphosarcoma of the breast. Surgery 1944; 16:836–853.
2. Smith RP, Vaughn JA. Sarcoma of the breast. Can Med Assoc J 1950; 62:584–589.
3. DeCosse JJ, Berg JW, Fracchia AA, et al. Primary lymphosarcoma of the breast. Cancer 1961; 15:1264–1268.
4. Jernstrom P, Sether JM. Primary lymphosarcoma of the mammary gland. JAMA 1967; 201:503–506.
5. Lawler MR, Richie RE. Reticulum sarcoma of the breast. Cancer 1967; 26:1438–1446.
6. Wiseman C, Liao KT. Primary lymphoma of the breast. Cancer 1972; 29:1705–1712.
7. Talvalkar GV. Primary lymphosarcoma of the breast. Indian J Cancer 1973; 10:322–329.
8. Mehrota RML. Lymphoma of the female breast. Indian J Pathol Pract 1974; 17:54–60.
9. Mambo NC, Burke JS, Butler JJ. Primary malignant lymphomas of the breast. Cancer 1977; 39:2033–2044.
10. Fischer MG, Chideckel NJ. "Primary" lymphoma of the breast. Breast 1984, 10:7–12.
11. Telesinghe PU, Anthony PP. Primary lymphoma of the breast. Histopathology 1985; 9:297–307.
12. Lamovec J, Jancar J. Primary malignant lymphoma of the breast. Cancer 1987; 60:3033–3041.
13. Brustein S, Kimmel M, Lieberman PH, et al. Malignant lymphoma of the breast. Ann Surg 1987; 205:144–150.
14. Schouten JT, Weese JL, Carbone PP. Lymphoma of the breast. Ann Surg 1981; 194:749–753.
15. American Joint Committee for Cancer Staging and End Results Reporting: Manual for Staging of Cancer. Chicago, 1978.
16. The Non-Hodgkin's Lymphoma Pathologic Classification Project: National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas. Cancer 1982; 49:2112–2135.
17. Jaffe ES: An overview of the classification of non Hodgkin's lymphomas. *In* Bennington JL, ed. Surgical Pathology of the Lymph Nodes and Related Organs, New York: WB Saunders, 1985, pp 135–145.
18. Cascinelli N, Singletary E, Greco M, et al. Long-term survival and prognostic factors for 2170 breast cancer patients treated at two cancer centers (Milan and Houston). Tumori 1989; 75:123–131.
19. Oberman HA. Primary lymphoreticular neoplasms of the breast. Surg Gynecol Obstet 1966; 123:1047–1051.
20. Freedman SI, Kagan AR, Friedman NB. Bilaterality in primary lymphosarcoma of the breast. Am J Clin Pathol 1971; 55:82–87.
21. Paulus DD. Lymphoma of the breast. Radiol Clin North Am 1990; 28:833–840.
22. Yi PI, Coleman M, Saltz L, et al. Chemotherapy for large cell lymphoma: a status update. Semin Oncol 1990; 17:60–73.
23. Carbone A, Volpe R, Tirelli U, et al. Primary lymphoblastic lymphoma of the breast. Clin Oncol 1982; 8:367–373.

Chronic ACE Inhibition Reduces Intimal Hyperplasia in Experimental Vein Grafts

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Intimal hyperplasia is an important factor in the pathophysiology of vein graft failure. Local renin-angiotensin systems recently have been shown to modulate the development of intimal hyperplasia in arteries after intimal injury. The effect of chronic angiotensin-converting enzyme (*ACE*) inhibition on the development of intimal hyperplasia in experimental vein grafts was examined in this study. Ten New Zealand White rabbits received 10 mg/kg of captopril daily in their drinking water. One week later the right carotid artery was divided and bypassed with the reversed right external jugular vein in these rabbits and in 10 matched controls. Captopril was continued for 28 days after operation, when all the grafts were harvested. Five grafts from each group were perfusion fixed, and the intimal thickness in the proximal, middle, and distal segments was determined. Rings from the remaining grafts ($n = 20$ in each group) were studied *in vitro* under isometric tension, and their responses to norepinephrine (NE), histamine (HIST), serotonin (5-HT), angiotensin I (AI), and angiotensin II (AII) was measured. The intimal thickness of the proximal, middle, and distal segments of the captopril-treated grafts were significantly less than controls, being reduced in all segments by approximately 40% ($p < 0.0001$). With regard to vasoreactivity, the captopril-treated grafts were hypersensitive to 5-HT (control ED$_{50}$ $5.5 \pm 0.5 \times 10^{-7}$ mol/L *vs.* captopril-treated $1.1 \pm 0.2 \times 10^{-6}$ mol/L; $p < 0.005$) although the maximal response was significantly reduced (control 1.6 ± 0.3 g *vs.* captopril-treated 0.8 ± 0.1 g; $p < 0.05$). There were no differences in sensitivity between control and captopril-treated rings with respect to NE, HIST, AI, or AII. Four of the ten captopril-treated segments, however, failed to respond to AI, and the maximal active tension of the responders was significantly reduced (control 0.47 ± 0.06 g *vs.* 0.20 ± 0.05 g; $p < 0.02$). These results suggest that ACE is involved in the modulation of vein graft intimal hyperplasia, and that ACE inhibitors may have therapeutic applications in patients undergoing vein bypass procedures.

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AUTOGENOUS SAPHENOUS VEIN is the preferred conduit for most arterial bypass procedures where the vessel sizes are compatible and no suitable arterial graft is available. Vein graft failure, however, remains a significant clinical problem.[1-3] The pathophysiology of vein graft failure has not been clearly elucidated. Autogenous vein grafts undergo structural and functional alterations after exposure to the arterial circulation. Intimal thickening develops in all veins within a short period after grafting. The pathologic significance of intimal thickening is uncertain, although some authors suggest that it may be a precursor to the development of atheroma and ultimate graft failure.[4-6] Localized regions of intimal hyperplasia have been associated with graft stenoses and may be harbingers of graft occlusion.[7]

The role of the renin-angiotensin system in the pathogenesis of atherosclerosis is unclear. There is, however, convincing evidence that local renin-angiotensin systems exist in many arteries and veins.[8,9] The activity of these systems results in the local production of angiotensin II independent of the systemic renin-angiotensin system. Angiotensin-converting enzyme (ACE) is a membrane-bound enzyme present in the endothelium of many large and medium-sized vessels in the peripheral circulation. It converts the relatively inactive angiotensin I (AI) to the potent vasoconstrictor angiotensin II (AII). The smooth muscle cells of these vessels have numerous AII receptors. AII has been shown to stimulate hypertrophy in cultured smooth muscle cells and to induce production of the proto-oncogenes c-*fos* and c-*myc*, as well as in the A chain of platelet-derived growth factor (PDGF).[10-12]

Local renin-angiotensin systems may be involved in

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the response of blood vessels to intimal injury occurring after angioplasty, endarterectomy, and vein grafting. Inhibition of ACE has been shown to significantly reduce intimal hyperplasia after balloon-catheter injury in rat carotid artery and to have antiatherogenic effects in heritable hyperlipidemic rabbits and cholesterol-fed monkeys.[13-15] The aims of the present study were to examine the effect of chronic *ACE* inhibition on the development of intimal hyperplasia and the vasomotor function of experimental vein grafts.

Materials and Methods

Experimental Design

Two groups of age and weight-matched adult male New Zealand White rabbits weighing 2 to 2.5 kg were studied. In 10 animals, captopril was administered in a dose of 10 mg/kg/day in the animals' drinking water; 10 additional animals were used as controls. One week later all animals underwent reversed autogenous vein bypass grafting of the right common carotid artery using the right external jugular vein. Blood pressure was measured at intervals during the study and the grafts were harvested after 28 days. Five grafts in each group were perfusion fixed for examination by light microscopy and the remainder studied *in vitro* under isometric tension. The grafts' responses to norepinephrine (NE), histamine (HIST), angiotensin I (AI), angiotensin II (AII), and serotonin (5-HT) were assessed. The animals received a normal diet during the period of study. Animal care complied with the "Principles of Laboratory Animal Care" and the "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 80-23, revised 1985).

Animal Operations

General anesthesia was induced and maintained for all operations using ketamine (60 mg/kg), acepromazine (1 mg/kg), and xylazine (5 mg/kg) intramuscularly. A single dose of prophylactic antibiotic in the form of 30,000 IU/kg of benzanthine and procaine penicillin (Durapen; Vedco, Inc., Overland Park, KS) was given intramuscularly at the time of induction. The right external jugular vein was harvested through a longitudinal neck incision as previously described.[16] The vein was stored in heparinized saline (5 IU/ml) while the common carotid artery was mobilized. The rabbit then was systemically heparinized (200 IU/kg IV), and a 4-cm section of the common carotid artery isolated between clamps. The vein was reversed and an end-to-side anastomosis was created with continuous 10-0 monofilament nylon suture (Ethicon, Inc., Somerville, NJ). The artery was ligated and divided between the anastomoses, leaving a graft of approximately 3 cm in length.

Four weeks later the animals were reanesthetized as described previously. The vein grafts from five animals in each group were left *in situ* and, after death, were fixed by perfusion and processed for histology. The vein grafts for *in vitro* study were removed atraumatically through longitudinal incisions and placed in oxygenated Krebs solution. Four rings, 4 to 5 mm in length, were cut from the central portion of each graft in preparation for isometric tension studies.

Blood Pressure Measurement

The arterial pressure of all animals was measured through a cannula placed in the middle ear artery using a catheter-tipped pressure transducer (Millar Instruments Inc., Houston, TX). Measurements were obtained before entry into the study, at the time of vein grafting, and at the time of harvest. Blood pressure was continuously monitored with the animal awake in a quiet environment and the pressure recorded when stable.

Histology

The grafts chosen randomly for histology were exposed under general anesthesia. The animal was systemically heparinized (500 IU/kg) and killed with an overdose of sodium pentobarbital. The thoracic aorta was exposed and cannulated and the graft perfused at the animal's last recorded blood pressure with normal saline followed by 10% formaldehyde. After 30 minutes' fixation *in situ,* the grafts were removed, fixed overnight by immersion, and divided into proximal, middle, and distal segments. The segments were paraffin sectioned at 5 μm and stained with modified Masson's trichrome and Verhoeff's elastic tissue stains.[17] The sections were examined by light microscopy in a blinded fashion. The intimal thickness was measured at 20 random points at even intervals around the circumference of each section using computerized videometric analysis (Innovision 150; American Innovision Inc., San Diego, CA); mean thickness for each graft segment was calculated from two separate sections.

In Vitro *Isometric Tension Studies*

Vein graft rings from control and captopril-treated animals were suspended from stainless steel hooks in an organ bath containing oxygenated Krebs solution of the following composition: 122 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L $MgCl_2$, 2.5 mmol/L $CaCl_2$, 15.4 mmol/L $NaHCO_3$, 1.2 mmol/L KH_2PO_4, and 5.5 mmol/L glucose. The solution was maintained at 37 C and bubbled with a mixture of 95% O_2 and 5% CO_2. One hook was fixed to the bottom of the bath and the other connected to a force transducer (Myograph F-60; Narco Bio-Systems, Houston, TX). Responses were recorded on a multichannel polygraph (Physiograph MK-111-S; Narco Bio-Systems). The optimal resting tension for each ring was determined by its maximal response to a modified oxygenated Krebs solution containing 60 mmol/L KCl, 66.7 mmol/L NaCl, 1.2 mmol/L $MgCl_2$, 2.5 mmol/L $CaCl_2$, 15.4 mmol/L $NaHCO_3$, 1.2 mmol/L KH_2PO_4, and 5.5 mmol/L glucose at resting tensions ranging from 0.4 to 2.5 g. The optimal resting isometric tension was applied and the tissue allowed to equilibrate in physiologic Krebs solution for 1 hour. The remainder of the experiments were performed at each ring's specific optimal resting tension.

Norepinephrine (NE; Sigma Chemical Co., St. Louis, MO), dissolved in 10^{-3} mol/L HCl, was added in a cumulative manner to each organ bath chamber and the responses were recorded. After washout and re-equilibration for 45 minutes, dose–response curves were similarly obtained in response to histamine (HIST), angiotensin I (AI), angiotensin II (AII), and serotonin (5-HT; all from Sigma; all dissolved in distilled H_2O). To avoid tachyphylaxis to either AI or AII, half the segments in each group were tested with only one of these agonists.

Statistics

The isometric responses of the rings were subsequently converted to percent maximal response to yield standard dose–response curves. The dose–response relationship was analyzed using logistic analysis to facilitate median effective dose (ED_{50}) calculation.[18] The maximal response of each ring was expressed as grams of isometric tension developed.

All data are expressed as the mean ± standard error of the mean. Differences in means were tested for significance using two-tailed Student's t test for unpaired data with p values < 0.05 regarded as significant.

Results

All animals survived the procedure and all grafts were patent at the time of harvesting. There was no significant difference in mean arterial pressure between the control and captopril-treated animals. The optimal resting tension for the control grafts (0.99 ± 0.06 g) was significantly greater than the optimal resting tension for the captopril-treated grafts (0.75 ± 0.01 g; $p < 0.001$).

Intimal Thickness

All grafts in both groups exhibited some degree of intimal hyperplasia consisting of smooth muscle proliferation, the formation of a neo-intima, and the deposition of collagen and elastin in both the intima and media (Fig. 1). Morphometrically determined intimal thickness of the

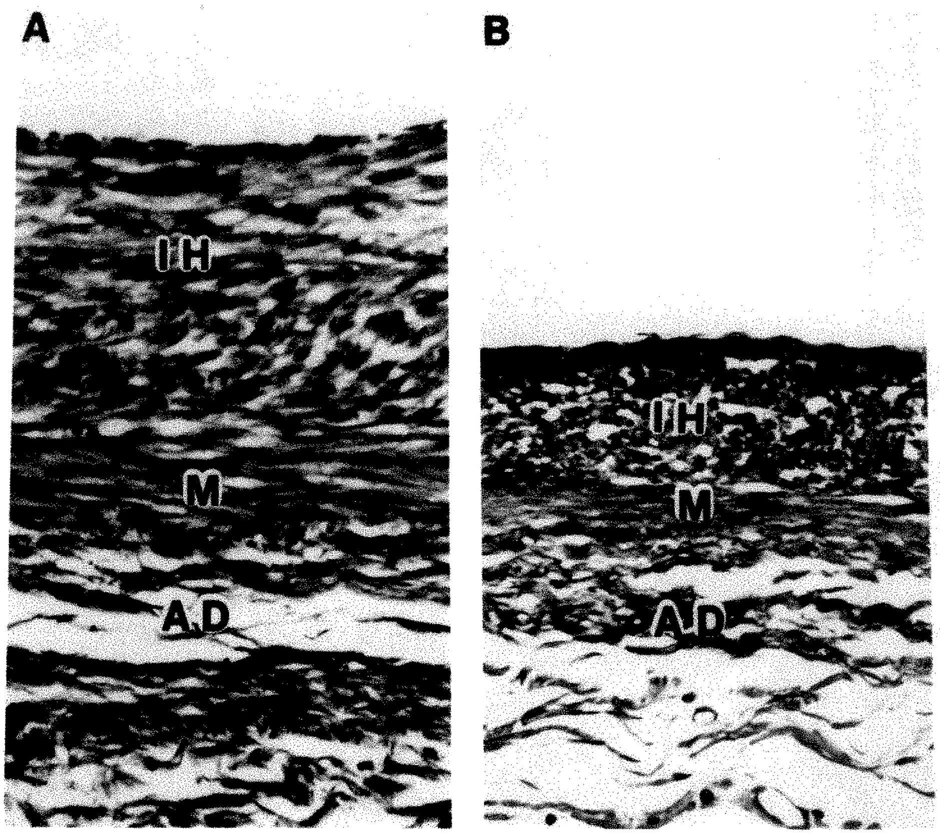

FIG. 1. A, Control vein graft showing the degree of intimal hyperplasia after 4 weeks. B, captopril-treated vein graft, with approximately 40% reduction in intimal thickness. Modified Masson's trichrome and Verhoeff's elastic tissue stains (IH, intimal hyperplasia; M, media; AD, adventitia). Original magnification ×200.

TABLE 1. *Effect of Captopril on Vein Graft Intimal Thickness*

Segment	Control Group (μm)	Captopril-treated Group (μm)	p
Proximal	156 ± 5	90 ± 4	<0.0001
Middle	150 ± 8	95 ± 5	<0.0001
Distal	151 ± 7	88 ± 3	<0.0001

grafts in the captopril-treated group was significantly less than controls in each of the proximal, middle, and distal segments (Table 1). Overall intimal thickness of the captopril-treated grafts was reduced by 40.3% as compared with controls.

In Vitro *Isometric Tension Studies*

With regard to vasomotor function, all grafts responded in classic "sigmoid" fashion to all agonists. The grafts from the captopril-treated animals were hypersensitive to *5-HT* (control ED_{50} $1.1 \pm 0.2 \times 10^{-6}$ mol/L *vs.* captopril-treated $5.5 \pm 0.5 \times 10^{-7}$ mol/L; $p < 0.005$, Fig. 2). The maximal response to *5-HT* in captopril-treated grafts, however, was significantly less than controls (control 1.6 ± 0.3 g *vs.* captopril-treated 0.8 ± 0.1 g; $p < 0.05$, Fig. 3).

Vein graft sensitivities to the other agonists are shown in Table 2. There were no significant differences in sensitivities in response to NE, HIST, AI, or AII. The captopril-treated group exhibited mild reductions in maximal tension to NE, HIST, and AII, although these reductions did not reach statistical significance (Fig. 3). Four of the 10 captopril-treated segments failed to respond to AI and, although there was no significant difference in the sensitivity of the remainder in terms of ED_{50} (Table 2), the maximal response was significantly reduced from 0.47 ± 0.06 g in the control group to 0.20 ± 0.05 g in the captopril-treated group ($p < 0.02$, Fig. 3).

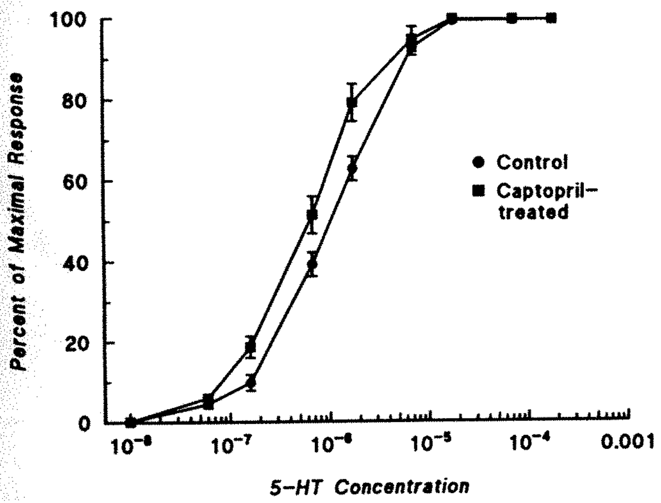

FIG. 2. 5-HT dose-response curves for control (●n = 5 animals, 20 rings) and captopril-treated vein grafts (■n = 5 animals, 20 rings). ED_{50} value was significantly reduced for the captopril-treated grafts (see text).

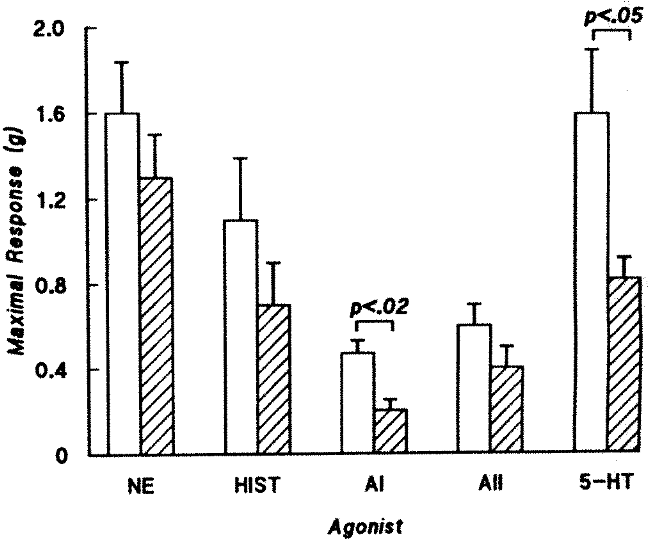

FIG. 3. Maximal response of control (open bars) and captopril-treated (hatched bars) vein grafts to norepinephrine (NE), histamine (HIST), angiotensin I (AI), angiotensin II (AII), and serotonin (5-HT). Significant reductions in maximal tension were noted with respect to AI ($p < 0.02$) and 5-HT ($p < 0.05$).

Discussion

The results of this study show that chronic inhibition of angiotensin-converting enzyme (ACE) with captopril significantly reduces the development of intimal hyperplasia in experimental vein grafts. The dose of captopril used, when corrected for body weight, was approximately twice that typically used in humans. It has been shown, however, that rabbits require up to 10 times the dose of captopril to achieve an equivalent degree of ACE inhibition generally attained in humans.[19] Thus the observed reduction in intimal hyperplasia was achieved with a dose of captopril equivalent to about 20% that typically used in patients. The relatively low dose of captopril employed herein is further illustrated by the fact that the mean arterial pressures of the control and captopril-treated groups were not statistically different.

The reduction in intimal hyperplasia was approximately 40% in all vein graft segments. This is, to our knowledge,

TABLE 2. *Vein Graft Sensitivity*

Agonist	Control Group (M)	Captopril-treated Group (M)	p
Norepinephrine	$7.8 \pm 2.5 \times 10^{-6}$	$7.9 \pm 2.8 \times 10^{-6}$	NS
Histamine	$4.0 \pm 1.0 \times 10^{-5}$	$3.4 \pm 1.2 \times 10^{-5}$	NS
Angiotensin I	$1.6 \pm 0.3 \times 10^{-7}$	$2.0 \pm 1.1 \times 10^{-7}$	NS
Angiotensin II	$3.1 \pm 0.7 \times 10^{-8}$	$2.6 \pm 0.7 \times 10^{-8}$	NS

the first demonstration of the ability of a systemically administered ACE inhibitor to attenuate intimal hyperplasia in vein grafts. A reduction in intimal hyperplasia has, however, been observed in other models. Neo-intima formation has been shown to be reduced up to 80% in intimally injured rat carotid arteries by administration of angiotensin-converting enzyme inhibitors.[13] The medial hyperplasia that has been observed in the vessels of spontaneously hypertensive rats also can be significantly reduced by ACE inhibition, whereas other anti-hypertensive agents are ineffective.[20] Lastly captopril has also been shown to have anti-atherogenic effects in heritable hyperlipidemic rabbits and cholesterol-fed monkeys.[14,15]

The mechanisms responsible for the reduction in intimal hyperplasia in this study are speculative. Captopril has a wide range of pharmacologic effects, including (1) ACE inhibition reducing local and systemic levels of angiotensin II, with consequent hypotension,[21] (2) free radical scavenging activity,[22] and (3) reduction of sympathetic nervous system activity,[21] and (4) anti-platelet activity.[23]

Angiotensin II has been shown to induce hypertrophy in cultured aortic smooth muscle cells,[10-12] and to augment proliferation of NIH 3T3 cells stimulated by epidermal growth factor.[24] The effect appears to be associated with increased expression of the proto-oncogenes c-*fos* and c-*myc* as well as the production of the A chain of PDGF. In addition the *mas* oncogene has been shown to encode a functional angiotensin II receptor.[25] Angiotensin II significantly increases DNA synthesis in cells transfected with this gene. Because experimental vein grafts have been shown to exhibit increased local ACE activity,[26] it is possible that smooth muscle cell proliferation could be locally mediated by angiotensin II and consequently reduced by ACE inhibition.

Captopril, unlike other ACE inhibitors, contains a sulfhydryl (SH) group and can act as a free radical scavenger.[22] Sulfhydryl-containing ACE inhibitors have been shown to protect cultured endothelial cells from free radical injury.[27] In addition the superoxide dismutase mimic, desferrioximine Mn^{3+}, has been shown to reduce intimal hyperplasia in the same experimental vein graft model used in this study.[28] Further studies employing non–sulfhydryl-containing ACE inhibitors may show if this mechanism is important in modulating intimal hyperplasia.

Captopril is a potent antihypertensive agent.[21] Many antihypertensive drugs have been shown to inhibit intimal hyperplasia or atherogenesis, including calcium antagonists, prazosin, and β-blockers.[29-31] The captopril dose used in this study did not significantly reduce blood pressure, however, so this mechanism is unlikely to be responsible for the observed effect. Captopril also has been shown to reduce sympathetic nervous system activity.[21] This effect could reduce effect the development of intimal hyperplasia because α-adrenoreceptor antagonism has been shown to reduce intimal hyperplasia in balloon-injured aorta.[30] Because there was no difference between control and experimental grafts' response to norepinephrine, however, and because these vessels are denervated in the process of grafting, it is unlikely that captopril's sympatholytic properties were involved.

Chronic ACE inhibition had several effects on the vasomotor function of vein grafts in this study. Vein grafts from captopril-treated animals exhibited decreased optimal resting tension as well as attenuated maximal active tension in response to AI and 5-HT. In addition the maximal contraction in response to all tested agonists (NE, HIST, AI, AII, and 5-HT) tended to be reduced (Fig. 3). The reduced optimal resting tension and maximal active tension probably reflect the reduction of smooth muscle cells in captopril-treated grafts.

The increased sensitivity to 5-HT in the experimental group was an unexpected finding. The importance of the interaction between 5-HT and the vascular system has only recently been recognized. Rabbit jugular vein does not constrict in response to 5-HT but, after grafting into the arterial circulation, develops 5-HT_2 receptor-mediated contraction.[32-34] The sensitivity of vein grafts to 5-HT does not change once developed despite significant increases in the degree of intimal hyperplasia.[33] There is no apparent explanation for the observed increase in vein graft 5-HT sensitivity after captopril treatment, although it is tempting to postulate that modulation of platelet function by captopril[23] could deprive the developing cells of a source of 5-HT, thereby inducing supersensitivity.

In summary the results of this study indicate that captopril can significantly reduce intimal hyperplasia and alter vasoreactivity in experimental vein grafts. This may be due to inhibition of smooth muscle proliferation by local renin-angiotensin systems or to free radical scavenging effects. The dose used was equivalent to 20% that typically prescribed for humans and did not significantly affect systemic blood pressure. It is concluded that ACE inhibitors may have therapeutic applications in patients undergoing vein bypass graft procedures.

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References

1. Grondin CM, Campeau L, Thornton JC, et al. Coronary artery bypass grafting with saphenous vein. Circulation 1989; 79(suppl 1): 1-24–1-29.
2. Loop FD, Lytle BW, Cosgrove DM, et al. Influence of the internal mammary-artery graft on 10 year survival and other cardiac events. N Engl J Med 1986; 314:1–6.
3. Kent KC, Whittemore AD, Mannick JA. Short-term and midterm

results of an all-autogenous policy for infrainguinal reconstruction. J Vasc Surg 1989; 9:107–114.

4. Myhre HO, Halvorsen T. Intimal hyperplasia and secondary changes in vein grafts. Acta Chir Scand 1985; Suppl 529:63–67.
5. Fuchs JC, Mitchener JS 3rd, Hagen PO. Postoperative changes in autologous vein grafts. Ann Surg 1978; 188:1–15.
6. Grunwald J, Chobanian AV, Haudenschild CC. Smooth muscle cell migration and proliferation: atherogenic mechanisms in hypertension. Atherosclerosis 1987; 67:215–221.
7. Berkowitz HD, Greenstein S, Barker CF, Perloff LJ. Late failure of reversed vein bypass grafts. Ann Surg 1989; 210:782–786.
8. Dzau VJ. Implications of local angiotensin production in cardiovascular physiology and pharmacology. Am J Cardiol 1987; 59: 59A–65A.
9. Rosenthal JH, Pfeifle B, Michailov ML, et al. Investigation of components of the renin-angiotensin system in rat vascular tissue. Hypertension 1984; 6:383–390.
10. Dzau VJ. Vascular renin-angiotensin: a possible autocrine or paracrine system in control of vascular function. J Cardiovasc Pharmacol 1984; 6:S377–S382.
11. Geisterfer AA, Peach MJ, Owens GK. Angiotensin II induces hypertrophy, not hyperplasia, of cultured rat aortic smooth muscle cells. Circ Res 1988; 62:749–756.
12. Naftilan AJ, Pratt RE, Eldridge CS, et al. Angiotensin II induces *c-fos* expression in smooth muscle via transcriptional control. Hypertension 1989; 13:706–711.
13. Powell JS, Clozel JP, Muller RKM, et al. Inhibitors of angiotensin converting enzyme prevent myointimal hyperplasia after vascular injury. Science 1989; 245:186–188.
14. Chobanian AV, Haudenschild CC, Nickerson C, Drago R. Antiatherogenic effect of captopril in the watanabe heritable hyperlipidemic rabbit. Hypertension 1990; 15:327–331.
15. Aberg G, Ferrer P. Effects of captopril on atherosclerosis in cynomolgus monkeys. J Cardiovasc Pharmacol 1990; 15(suppl 5): S65–S72.
16. Cross KS, el Sanadiki MN, Murray JJ, et al. Mast cell infiltration: a possible mechanism for vein graft vasospasm. Surgery 1988; 104:171–177.
17. Goodfellow BS, Mikat EM. A stain for the simultaneous demonstration of collagen, muscle, and elastic elements in mammalian tissue. Lab Med 1988; 19:243–244.
18. Finney DJ. Quantal responses and tolerance distribution. *In* Finney DJ, ed. Statistical Methods and Biological Assay. London: Charles Griffin & Co., Ltd., 1978, pp 347–369.
19. Harrigan JR, Meredith PA, Reid JL. Interspecies differences in in vitro *ACE* inhibition (abstract). Proceedings of the Fourth European Meeting on Hypertension, 1989.
20. Clozel JP, Hefti F. Effects of chronic therapy with cilazopril, a new long acting angiotensin converting enzyme inhibitor, on regional blood flow in conscious spontaneous hypertensive rats. J Cardiovasc Pharmacol 1987; 10:350–355.
21. Brogden RN, Todd PA, Sorkin EM. Captopril. An update of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in hypertension and congestive heart failure. Drugs 1988; 36:540–600.
22. Chopra M, Scott N, McMurray J, et al. Captopril: a free radical scavenger. Br J Clin Pharmacol 1989; 27:396–399.
23. Someya N, Morotomi Y, Kodama K, et al. Suppressive effect of captopril on platelet aggregation in essential hypertension. J Cardiovasc Pharmacol 1984; 6:840–843.
24. Norman J, Badie-Dezfooly B, Nord EP, et al. EGF induced mitogenesis in proximal tubular cells: potentiation by angiotensin II. Am J Physiol 1987; 253:F299–F309.
25. Jackson TR, Blair LAC, Marshall J, et al. The mas oncogene encodes an angiotensin receptor. Nature 1988; 335:437–440.
26. O'Donohoe MK, Radic ZS, Schwartz LB, et al. Increased local activity of angiotensin converting enzyme in experimental vein grafts (abstract). FASEB J 1990; 4:A903–A903.
27. Mak T, Dickens BF, Weglicki WB. Hydroxyl radical scavenging and attenuation of free radical injury in endothelial cells by SH-containing *ACE* inhibitors (abstract). Circulation 1989; 80(Suppl II): II-242–II-242.
28. Schuman RW, Cross KS, el Sanadiki MN, et al. Reduction of graft intimal hyperplasia by a free radical scavenger (abstract). Circulation 1988; 78(suppl II):II452.
29. Diccianni MB, Cardin AD, Britt AL, et al. Effect of sustained release formulation of diltiazem on the development of atherosclerosis in cholesterol-fed rabbits. Atherosclerosis 1987; 65:199–205.
30. O'Malley MK, McDermott EW, Mehigan D, O'Higgins NJ. Role for prazosin in reducing the development of rabbit intimal hyperplasia after endothelial denudation. Br J Surg 1989; 76:936–938.
31. Chobanian AV, Brecher P, Chan C. Effect of propranolol on atherogenesis in cholesterol-fed rabbits. Circ Res 1985; 56:755–762.
32. el Sanadiki MN, Cross KS, Murray JJ, et al. Reduction of intimal hyperplasia and enhanced reactivity of experimental vein bypass grafts with verapamil treatment. Ann Surg 1990; 212:87–96.
33. Radic ZS, O'Donohoe MK, Schwartz LB, et al. Alterations in serotonergic receptor expression in experimental vein grafts. J Vasc Surg 1991; 14:40–47.
34. Makhoul RG, Davis WS, Mikat EM, et al. Responsiveness of vein bypass grafts to stimulation with norepinephrine and 5-hydroxytryptamine. J Vasc Surg 1987; 6:32–38.

Late Iliac Artery Aneurysms and Occlusive Disease After Aortic Tube Grafts for Abdominal Aortic Aneurysm Repair

A 35-Year Experience

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Controversy continues over whether patients treated with straight Dacron aortic tube grafts for an abdominal aortic aneurysm remain at significant risk for subsequent development of iliac aneurysm or occlusive disease. To address this issue, the authors performed a population-based analysis of 432 patients who had an abdominal aortic aneurysm diagnosed between 1951 and 1984. Aneurysm repair was performed eventually in 206 patients (48%). To ascertain differences in late development of graft-related complications, iliac aneurysms, and arterial occlusions, the authors compared all tube-graft patients with similar numbers of bifurcated-graft patients matched for age and year of operation. In the tube-graft group, no subsequent clinically evident or autopsy-proven iliac aneurysms or iliac occlusive disease were noted. Over a mean follow-up of 6 years (range, 4 to 18 years), new aortic aneurysms occurred in the proximal aorta in both tube and bifurcated-graft patients (5.0% and 2.5%, respectively). In contrast the cumulative incidence of graft-related complications was higher with a bifurcated prosthesis (12.8%) compared with a straight graft (5.0%) ($p = 0.15$). These problems generally occurred 5 to 15 years postoperatively and emphasize the need for long-term graft surveillance. The authors conclude that straight tube-grafts for repair of abdominal aortic aneurysms provide excellent late patency with minimal risk of subsequent iliac aneurysm development.

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ABDOMINAL AORTIC ANEURYSMS (AAA) often do not initially involve the common iliac arteries. In such patients straight aortic tube grafts can be performed. Some controversy continues, however, over whether the common iliac arteries will eventually develop aneurysmal or occlusive disease. In an earlier study based primarily on our referral population, we found a few instances of late iliac aneurysm disease in patients treated with tube grafts and questioned whether bifurcated prostheses should be routinely used in all patients with abdominal aortic aneurysms.[1] Other authors have not found these same problems and advocate the use of a straight aortic prosthesis when intraoperative findings deem it appropriate.[2-6]

To address this controversy, we have reviewed 432 residents of Rochester, Minnesota, who have had aortic aneurysms diagnosed between 1951 and 1984. This community-based study minimizes bias inherent in a referral population and allows complete long-term follow-up of patients.

Methods

The diagnosis made and surgical procedures performed on Rochester residents (60,000) by essentially all medical care providers are indexed and the original medical records can be retrieved readily for review. The index covers care delivered at the Mayo Clinic and its two large affiliated hospitals (St. Marys and Rochester Methodist) as well as other institutions serving the community, including Olmsted Medical Group, the Olmsted Community Hospital, the Rochester State Hospital, the Veterans Administration, and the University of Minnesota Hospitals in Minneapolis, and other hospitals and practitioners in the vicinity. These indices incorporate the diagnosis made among outpatients seen in office or clinic consultations, emergency room visits, house calls, nursing home visits, as well as diagnosis recorded for hospital inpatients, on

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death certificates, and at autopsy. The potential of this data retrieval system for population-based studies has been described previously.[7]

All residents of Rochester with an abdominal aortic aneurysm newly diagnosed from 1951 to 1984 were identified. Because the purpose of this study was to assess late results, patients not surviving the 30-day perioperative period were not studied. All patients who underwent successful repair with a straight aortic prosthesis were selected for careful review as well as a group of patients with bifurcated aortic grafts matched for age and year of operation with the group of tube graft patients. Presenting factors of interest that were recorded were age, sex, and presence of rupture (Table 1). From the operating and anesthetic records, the operating time and amount of blood transfused was noted as well as the surgeon's reason for selecting the particular type of graft. Subsequent clinical history was reviewed to determine the late survival and subsequent symptomatic occlusive or aneurysmal disease. After graft placement, periodic (2 to 5 years) abdominal ultrasound or computed axial tomography (CAT) scanning has been performed on patients in our community. Any subsequent vascular operations for aneurysmal or occlusive disease were noted. Overall survival and survival free of graft and nongraft vascular complications were assessed using Kaplan-Meier[8] survival analysis and the log-rank test.[9] Operative time and blood usage were compared between groups with Wilcoxon rank-sum test.[10]

Results

Of the 432 patients diagnosed with abdominal aortic aneurysm, 206 (48%) underwent eventual repair of their abdominal aortic aneurysms. The remaining patients had smaller aneurysms (generally <5 cm in diameter) that have been followed with serial ultrasounds and repaired selectively.

Tube Grafts

Thirty-nine patients underwent successful aneurysm repair with a straight graft. Average follow-up for these 39 patients was 6 years. Twenty-six patients have died, with a 5-year survival of 58% (Fig. 1). The causes of death are listed in Table 2. Autopsies and clinical records of these patients did not demonstrate any iliac aneurysms or iliac occlusive disease. The 13 patients treated with tube grafts who remain alive have had no further surgery for iliac aneurysmal or occlusive disease. Two patients (5%) in this group did have graft-related complications, one disruption at the proximal anastomosis 1 month postoperatively and the other a false aneurysm involving the proximal anastomosis occurring 15 years postoperatively (Table 3). Two additional patients developed aneurysmal disease proximal to the aortic graft, and one patient was noted to have left leg claudication with intact femoral pulses 15 years postoperatively (Table 4). It does not appear that the outcome of these patients would have been different had a bifurcated graft been chosen over a tube graft. The average operating time and amount of intraoperative blood transfusion are listed in Table 5.

TABLE 1. *Clinical Characteristics of Rochester, Minnesota, Residents who Underwent Successful Abdominal Aortic Tube Graft Placement and a Sample of Rochester Residents Matched by Age and Year of Surgery who Underwent Bifurcated Graft Placement, 1951–1984*

	Type of Graft	
Characteristic	Tube (n=39)	Bifurcated (n= 39)
Age ≥ 70 yr	49%	51%
Male	72%	82%
Ruptured at time of surgery	21%	10%

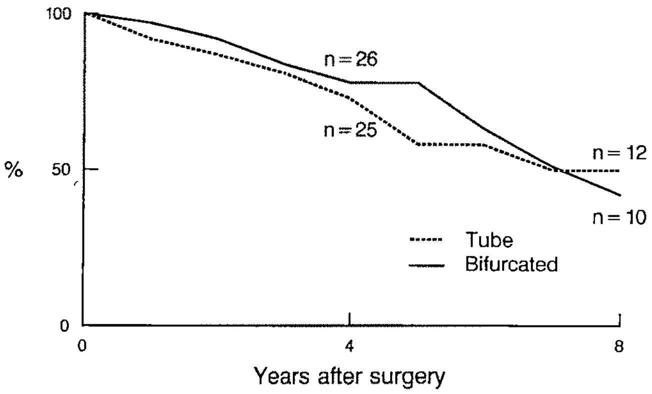

FIG. 1. Survival of patients undergoing tube *versus* bifurcated Dacron grafts for abdominal aortic aneurysms, matched for age and year of operation, Rochester, Minnesota, 1951–1984.

Bifurcated Grafts

The reasons for choosing a bifurcated graft in the 39 matched patients were iliac aneurysms (59%), iliac occlusive disease (21%), and no reason was given in 21%. The

TABLE 2. *Causes of Late Death Among Rochester, Minnesota, Residents who Underwent Tube and Bifurcated Aortic Graft, 1951–1984*

	Type of Graft	
Cause of Death	Tube (n=22/39)	Bifurcated (n=22/39)
Cardiac	9	7
Stroke	2	3
Renal failure	2	0
Anastomotic rupture	1	1
Thoracic dissection	1	0
Other	7	11

TABLE 3. *Complications Related to Graft Among Rochester, Minnesota, Residents who Underwent Tube (n=39) and Bifurcated (n=39) Aortic Graft Placement, 1951–1984*

	Type of Graft	
Complications Related to Graft	Tube	Bifurcated
Rupture of proximal anastomosis	1 (1 mo)	1 (6 yr)
Rupture of distal anastomosis	0	1 (16 yr)*
False aneurysm of proximal anastomosis	1 (15 yr)	0
False aneurysm of distal anastomosis	0	2 (4, 8 yr)†
Lymphocele	0	1 (2 mo)†

* Iliac.
† Femoral.

average follow-up in this group was also 6 years. Twenty-two patients have died, with a 5-year survival of 78% (Table 2 and Fig. 1). The overall survival was not significantly different than in the tube group (Fig. 1). Five patients (12.8%) in the bifurcated group had problems related to their grafts. There was one lymphocele in the groin that required drainage 2 months postoperatively. Two patients developed distal femoral anastomotic aneurysms at 4 and 8 years postoperatively. Dehiscence of a suture line was suspected in a patient who died in shock 6 years postoperatively. In a fifth patient, a left graft limb dehiscence was repaired 16 years postoperatively (Table 3). When compared with the tube group, there was a trend (p = 0.15) of more graft-related complications in the bifurcated group (Fig. 2).

Patients with bifurcated grafts also tended to have more late vascular problems that were *not* related to the graft (Table 4). Seven patients (17.9%) developed occlusive or aneurysmal disease distant to the aortoiliac segment. Two patients were noted to have claudication treated conservatively 3 and 6 years postoperatively. One patient required patch of the profunda femoris artery as well as a femoropopliteal bypass graft 4 months postoperatively for occlusive symptoms, and one patient required extension of the limb of the graft onto the superficial femoral and profunda femoris arteries for occlusive disease 6 years postoperatively. A fifth patient was noted on sonogram to develop a 3.5 cm proximal aortic aneurysm 6 years postoperatively, and a left iliac artery aneurysm was noted at the time of another surgical procedure in a sixth patient 14 years postoperatively. One patient required repair of a descending thoracic aortic dissection 4 years after the original aortic surgery (Table 4). The median operating time was significantly greater in the bifurcated graft group ($p < 0.001$). There was no difference in intraoperative blood transfusion (Table 5).

TABLE 4. *Vascular Problems not Related to Graft Among Rochester, Minnesota, Residents With Tube or Bifurcated Aortic Graft Placed, 1951–1984*

Vascular Problems not Related to Graft	Type of Graft	
	Tube	Bifurcated
Proximal aneurysm	2 (8, 13 yr)*	1 (6 yr)
Descending thoracic dissection	0	1 (4 yr)
Iliac aneurysm	0	1 (14 yr)
Claudication	1 (15 yr)	2 (3, 6 yr)
Severe ischemia	0	2 (4 mo, 6 yr)

* Time interval from graft placement to vascular problem in parenthesis.

TABLE 5. *Median Operating Time and Median Number of Units of Blood Transfused by Type of Aortic Graft Among Rochester, Minnesota, Residents With Tube or Bifurcated Graft Placed, 1951–1984*

	Type of Graft (Median IQR)	
	Tube	Bifurcated
Operating time (min)	240 (210–255)	328 (300–406)
No. of units of blood transfused	4.0 (3–4)	4.0 (3–5)

IQR, interquartile range (25th–75th percentile).

Discussion

We were impressed with the low cumulative risk of late problems associated with use of tube grafts for AAA repair in our defined study population. This study group offered certain advantages over our selected referral practice in identifying late graft-related complications and new aneurysm and occlusive disease.[1,7] The first advantage was inclusion of our entire community-based experience to avoid the potential selection bias in our larger referral practice. Second the close and complete long-term follow-up of these patients allowed detection of all clinically apparent or autopsy-proven aneurysm or occlusive disease. Finally we have carefully matched *all* tube-graft patients with similar bifurcated-graft patients to attain a more accurate long-term comparison of graft-related and new aneurysm problems.

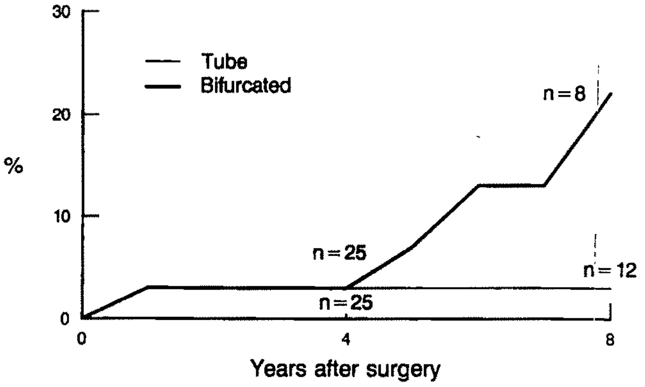

FIG. 2. Cumulative incidence of graft-related complications in patients undergoing tube *versus* bifurcated Dacron grafts for abdominal aortic aneurysms, matched for age and year of operation, Rochester, Minnesota, 1951–1984.

Our study, however, had several limitations. The first was a relatively low number of tube grafts compared with other studies that review an institution-wide experience. Thus our estimates of the risk of complications may be relatively imprecise: at 7 years 12.5% with bifurcated grafts (95% confidence interval (CI) = 0 to 26.4) and 2.6% with tube grafts (95% CI = 0 to 7.4). Relying solely on clinical presentations of problems or autopsy evidence of aneurysmal or occlusive disease also could have resulted in failure to detect some of these problems. The use of CAT scans or sonograms in long-term follow-up was selective in patients between 1951 and 1984. Generally patients with grafts underwent an abdominal ultrasound or CAT scan every 2 to 5 years after graft placement. If these tests missed any new aneurysms, autopsy also did not disclose any graft complications or aneurysms that we had not recognized clinically before death.

In addition to few late iliac aneurysms or occlusions, the potential benefits of tube grafts also include less periaortic dissection. This may reduce blood loss and lessen the likelihood of injury to associated structures such as iliac veins or ureters. In addition less disruption of periaortic parasympathetic and sympathetic nerve fibers may minimize postoperative sexual dysfunction. Another benefit is only two anastomoses instead of three. Conversely the main technical disadvantage of a tube graft is the difficulty of the distal anastomosis when the aortic bifurcation is very diseased or calcified.

Review of the literature confirms our finding that tube grafts can be employed with excellent long-term success. In two separate studies, Orr[2] and Snellen et al.[3] were able to use tube grafts in 50% of their patients with aortic aneurysms. They found that tube grafts were associated with less intraoperative blood loss and less operating time, especially in those patients with ruptured or leaking aneurysms. In the study by Snellen and co-workers, only 1 patient of 110 treated with tube grafts required further surgery for iliac stenosis. In two additional studies by van Vroonhoven[4] and Glickman et al.,[5] tube grafts also were used approximately 50% of the time. Van Vroonhoven also noted less operative time and intraoperative blood loss with the use of a tube graft. In 86 patients treated with tube grafts by Glickman and co-workers during an average follow-up of 5 years, only two patients required further bypass grafting to the femoral level. In another report, Nash[6] used tube grafts in 43 consecutive patients even if the iliac arteries appeared ectatic at the time of operation. Although the average follow-up was only 18 months, he reported no further surgery for subsequent iliac disease. More recently Provan et al.[11] have confirmed by serial postoperative CT scans that tube grafts can be used without significantly increasing subsequent need for a second operation for late iliac aneurysm development.

Of interest was the higher trend of subsequent graft-related complications as well as new aneurysmal or occlusive disease in the patients who received bifurcated grafts. These findings certainly do not implicate causality with the use of bifurcated grafts, but may indicate that the bifurcated grafts were performed in patients with more advanced arterial disease.

The late development of graft-related problems, new aneurysms, and occlusive disease emphasizes the need for careful late follow-up of all aneurysm patients whether treated with a tube or a bifurcated graft. To identify new aneurysms, we currently recommend periodic (every 2 to 5 years) chest radiographs as well as an aortic and popliteal ultrasound examination after abdominal aortic graft placement. The occasional development of proximal suprarenal aneurysmal disease suggests that periodic CT scanning may be warranted because chest radiographs and ultrasound may not clearly visualize the aorta adjacent to the diaphragm.

This study challenges previous concerns in referral studies that tube grafts for abdominal aortic aneurysms eventually require revision for late iliac aneurysms in a significant number of patients. Aortic tube grafts remain sufficient for patients with relatively normal iliac arteries at the initial AAA repair. Bifurcated aortic grafts should be reserved for patients with AAAs associated with *concurrent* iliac aneurysms or aorto-ileofemoral occlusive disease.

Acknowledgments

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References

1. Plate G, Hollier LH, O'Brien PC, et al. Recurrent aneurysms and late vascular complications following repair of abdominal aortic aneurysms. Arch Surg 1985; 120:590–594.
2. Orr WM, Davies M. Simplified repair of abdominal aortic aneurysms using nonbifurcated (straight) inlay prosthesis. Br J Surg 1974; 61:847–849.
3. Snellen JP, Terpstra OT, VanUrk H. The use of a straight tube graft decreases blood loss and operation time in patients with an abdominal aortic aneurysm. Neth J Surg 1984; 36:45–47.
4. van Vroonhoven TH JMV. Tube-inlay graft for abdominal aortic aneurysms. Arch Chir Neerl 1978; 30:164–168.
5. Glickman MH, Julian CC, Kimmins S, Evans WE. Aortic aneurysm: to tube or not to tube. Surgery 1982; 9:603–605.
6. Nash T. Abdominal aortic aneurysm: experience with the use of the straight tube method of treatment. Med J Aust 1975; 2:85–88.
7. Kurland LT, Molgaard CA. The patient record in epidemiology. Sci Am 1981; 245:54–63.
8. Kaplan EL, Meier P. Non-parametric estimation from incomplete observations. J Am Stat Assoc 1958; 53:457–481.
9. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 1966; 50:163–170.
10. Hollander M, Wolfe DA. Nonparametric Statistical Methods. New York: John Wiley & Sons, 1973, pp 68–75.
11. Provan J, Fialkov JA, Ameli FM, St. Louis EL. Is "tube" prosthetic repair of abdominal aortic aneurysm followed by postoperative dilatation of the common iliac arteries? Can J Surg (in press).

Can Doppler Pressure Measurement Replace "Exclusion" Arteriography in the Diagnosis of Occult Extremity Arterial Trauma?

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Although highly accurate, contrast arteriography is a costly, invasive, and time-consuming method to rule out occult arterial damage in injured extremities. Accordingly the authors assessed the sensitivity and specificity of Doppler-derived arterial pressure measurements in trauma victims undergoing evaluation for possible extremity arterial damage. Arterial pressure index (API) was calculated (Doppler arterial pressure distal to injury/Doppler arterial pressure in uninvolved arm), but not used in clinical decision making in 100 consecutive injured limbs in 93 trauma victims. All patients then underwent contrast arteriography. Twenty limbs had an API < 0.90 and an abnormal arteriogram, whereas 75 had both a normal API and a normal contrast study. One limb had a significant angiographic abnormality with an API > 0.90; two others had API < 0.90 but normal arteriograms. Two limbs with a normal API had false-positive arteriograms. When compared with arteriography, an API < 0.90 had a sensitivity of 87% and a specificity of 97% for arterial disruption in this series. Sensitivity and specificity rose to 95% and 97% when API was compared with clinical outcome. In the absence of obvious signs of arterial injury, API may be a reasonable substitute for screening arteriography in the traumatized extremity, particularly if close follow-up observation can be assured.

ARTERIOGRAPHY HAS SEVERAL functions in the trauma victim with penetrating or blunt injuries to the extremity. Few dispute its use in patients with clear evidence for an arterial injury (arterial bleeding, rapidly expanding hematoma, distal ischemia); here the goal of the arteriogram is precise localization of the level of arterial injury.[1,2] Similarly, "completion" arteriography is frequently useful after an arterial reconstruction to exclude a technical defect (intimal flap, suture line stenosis) that might lead to early postoperative thrombosis of the repair.[3]

More controversial is the widespread establishment of arteriography as a screening technique to exclude the presence of significant arterial injury in asymptomatic trauma victims. This practice has become particularly common in so-called "proximity" injuries of the extremities, where (despite the absence of "hard" signs of arterial injury) the injuring mechanism—a nearby gunshot or stab wound, or a fracture or dislocation—might have resulted in occult arterial damage.[1,2,4]

Recently several reports have pointed out that such "screening" or "exclusion" arteriography is rarely positive when wound proximity is the *only* indication for the study.[4-11] Further other studies have suggested that arteriographically demonstrated lesions previously considered worrisome generally have a benign prognosis and may only rarely necessitate operative intervention.[8,10,11]

Because physical examination alone is insufficiently sensitive and specific to rule out arterial disruption, a screening technique with diagnostic accuracy greater than that of physical examination, yet less invasive, costly, and time-consuming than contrast arteriography, would be ideal. We hypothesized that Doppler arterial pressure measurements, so reliable in the quantitation of *chronic* arterial disease, might be a potentially valuable screening tool in the trauma victim. Accordingly we designed a prospective study to investigate the accuracy of Doppler arterial pressure measurements in diagnosing the presence or absence of occult arterial injury in trauma victims with "proximity" extremity injuries.

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Patients and Methods

Trauma victims with blunt or penetrating extremity trauma (including all injuries between the neck and the wrist, and between the inguinal ligament and the ankle) were evaluated in the Emergency Department (ED) of Harborview Medical Center, Seattle. Each underwent, besides standard history, physical examination, and baseline laboratory examinations, measurement of Doppler systolic arterial blood pressure at the ankle or wrist, distal to an injury thought to threaten the extremity's artery. Systolic blood pressure was recorded as well in an uninvolved arm. An arterial pressure index (API) was computed as follows:

$$\text{API} = \frac{\text{Doppler systolic pressure in injured extremity}}{\text{Doppler arterial pressure in uninvolved arm}}$$

Each patient then underwent contrast arteriography in the angiographic suite (in all cases using a transfemoral approach, the Seldinger technique, an automated dye injector, and biplane images). Depending on clinical and arteriographic findings at the time, patients underwent immediate or delayed operation or in-patient observation, with follow-up scheduled in the vascular clinic in all cases.

Data collected included: identification, gender, age, wounding mechanism, results of pulse examination, API, Doppler signal characteristics, results of arteriography (including arteriographic findings, hospital charges, and time consumed by each study), results of intervention (operative or otherwise), and outcome during subsequent follow-up. Arterial pressure index > 0.90 was set as normal. Sensitivity, specificity, predictive values of "positive" and "negative" API, and overall accuracy were calculated by standard means, using both arteriographic findings and clinical outcome as the comparison standard (although comparison against clinical outcome was understood to be only as valid as the completeness of patient follow-up). Patients undergoing contrast arteriography solely to localize the site of an obvious arterial injury were excluded from this analysis.

Results

One hundred consecutive injured limbs in 93 trauma victims fulfilled study criteria—API, contrast arteriography, and documented follow-up. As noted in Table 1, the study population was composed almost entirely of young men, about three quarters of whom had suffered penetrating trauma.

TABLE 1. *Study Population*

Characteristic	n
Patients	93
M	86
F	7
Age (range, 11–62)	26.2
Injuries	100
Gunshot wound	58
Stab wounds	16
Fractures/dislocations	22
Other	4

Among these 100 injured limbs, arteriographic findings led to an intervention in 14 cases, including nine arterial reconstructions, two fasciotomies, and one therapeutic embolization. Arteriograms in two patients were interpreted as abnormal enough to warrant operation, but vessel exploration and intraoperative arteriography showed no abnormalities. Eighty-six arteriograms were read as normal or only minimally abnormal, findings that (when combined with the clinical setting) resulted in observation only.

Seventy-five limbs with normal arteriograms had API > 0.90. Similarly 20 limbs with significant arteriographic abnormalities had an API < 0.90. Two limbs had API < 0.90 but normal arteriograms. Three limbs had API > 0.90 but abnormal arteriograms. Among this last group of patients, one contrast study showed a small pseudoaneurysm of a secondary branch of the profunda femoris artery. The other studies with a "false-negative" API were the two limbs, previously mentioned, in which arteriography was abnormal but subsequent operative exploration showed no vessel injury. In fact, these were "false-positive" arteriograms.

One patient with a gunshot wound to the popliteal fossa initially had an API > 0.90 and a contrast arteriogram considered only minimally abnormal on both AP and lateral views. He was discharged: during a clinic visit 5 days later, however, he was noted to have a pulsatile mass in the popliteal fossa. His API had fallen to 0.76, and on repeat arteriography he was found to have a large pseudoaneurysm, subsequently repaired without complication using an interposed vein graft.

Compared with contrast arteriography, use of API < 0.90 to define an occult arterial injury resulted in 75 true negatives, 20 true positives, 2 false positives, and 3 false negatives. Using ultimate clinical outcome as the standard, these values were as follows: 77 true negatives, 20 true positives, 2 false positives, and 1 false negative.

With arteriographic findings for comparison, API < 0.90 had a sensitivity of 87% and a specificity of 97% for arterial disruption. Positive and negative predictive values were 91% and 96%, and overall accuracy was 95%. Using clinical outcome as the standard (thereby eliminating the confounding effect of the two false-positive arteriograms), sensitivity and specificity of API < 0.90 for arterial disruption rose to 95% and 97.5%, positive and negative predictive values to 91% and 99%, and overall accuracy to 97% (Table 2). (When compared against clinical outcome, contrast arteriography had a sensitivity and specificity of 100% and 97.5%, positive and negative predictive values of 91% and 100%, and an overall accuracy of 98%).

The hand-held Doppler device (Medasonics, Mountain View, CA) used in the Harborview Medical Center ED costs approximately $500; one is purchased every 4 years. Measuring Doppler limb pressures and computing API requires less than 10 minutes. Costs of arteriography in this study included mean hospital charges of $788 (range, $466 to $1486), and a mean of 2.4 hours expended (range, 0.8 to 4.2 hours). Aside from the two false-positive arteriograms, no technical complications or contrast toxicity could be ascribed to these 100 arteriograms.

Comment

Exploration is mandatory when blunt or penetrating extremity injury is accompanied by clear evidence for arterial disruption (significant arterial bleeding, distal ischemia); contrast arteriography is indicated in this setting only if required to localize the level and perhaps the magnitude of the arterial injury.[1,2,4] Some also believe that shotgun injuries uniformly warrant arteriography in view of these wounds' high likelihood of multiple occult vessel injuries.[9,12]

For wounds unaccompanied by gross signs of arterial damage, but worrisome for contiguity of the wounding mechanism to nearby vessels ("proximity" injuries), physical examination alone appears not to be sensitive or specific enough to discover all occult vascular injuries. Experience has documented, for example, that pulses may be palpated distal to complete arterial disruption[1,2,9]; a 9% incidence of major arterial disruption was demonstrated among 173 trauma victims said to have "normal" pulses on initial evaluation.[6] Conversely a traumatized limb may have normal perfusion, yet edema, hematoma, dressings, or splints may prevent accurate pulse palpation.

Routine operative exploration was once believed indicated in the setting of blunt or penetrating extremity trauma when clinical suspicion for an occult arterial injury was high. This approach was found to be rarely productive and needlessly invasive,[13] however, and contrast arteriography (either by formal means in the angiography suite[1,2,4] or in the ED or operating room[14]) has become the "gold standard" in evaluating occult arterial damage during the past decade.

TABLE 2. *Accuracy of Arterial Pressure Index < 0.90 Compared with Arteriography and Clinical Outcome*

Variable	Arteriography	Clinical Outcome
Sensitivity	0.87	0.95
Specificity	0.97	0.97
Positive predictive value	0.91	0.91
Negative predictive value	0.96	0.99
Overall accuracy	0.95	0.97

Recent studies have repeatedly noted the paucity of positive arteriograms in patients with few or no signs of arterial disruption, however. For example, Reid et al.[5] reviewed 534 "exclusion" arteriograms performed for injury proximity alone. Thirty-six arteriograms (6.7%) had significant findings, and only 19 patients (3.6% of the total) underwent an operative repair—none for threatened limb loss. Similarly during a review of 280 angiographic studies for trauma, Rose and Moore[6] describe 97 patients undergoing angiography solely for proximity of injury. None (0%) of these latter patients had a major vascular injury. Their study results led these authors to suggest that arteriography is not warranted for the indication of proximity of injury alone.[6] Other studies also have stressed how infrequently "screening" arteriograms will yield clinically relevant data.[4,7-11] And a vexing incidence of false-negative or false-positive arteriograms, in up to 5% of all cases, may lead to inappropriate therapeutic decision making,[5] further complicating the decision-making picture.

Various defects (intimal flaps, spasm, pseudoaneurysms, arteriovenous fistulas) found during such arteriographic studies in trauma victims were until recently an automatic indication for vessel exploration.[1,2,4] Studies by Stain et al[10] and Frykberg and colleagues,[8,11] however, have suggested that most such lesions arising from blunt or penetrating trauma follow a benign course, rarely requiring operation. Thus arteriography also appears to be overly sensitive; in other words, the technique may discover lesions that are clinically irrelevant.

Because contrast arteriography is expensive, time consuming, potentially toxic, and rarely productive in the evaluation of in injured limbs without obvious signs of vascular impairment, we sought to substitute an equivalently accurate but rapid, simple, inexpensive, noninvasive, and reproducible alternative as a screening tool in this setting. Impressed with the established accuracy of Doppler ankle pressure measurements in identifying significant *chronic* arterial occlusive disease, we set out to test the hypothesis that Doppler pressure measurements might be predictive of *acute* arterial damage in patients with injured limbs.

Although some studies[9,15] have questioned the reliability of Doppler pressures in extremity trauma, a recent editorial in the *Journal of Bone and Joint Surgery* supports the use of this technique in the trauma setting.[16] We also found such measurements to be highly accurate; in fact our study suggests that Doppler arterial pressure measurements may be virtually as reliable as arteriography. Arterial pressure index was normal, and thus failed to "uncover," a small profunda femoris artery pseudoaneurysm in one patient; conversely, false-positive arteriograms (in the presence of normal Doppler pressure measurements) resulted in nonproductive vessel explorations in two other patients.

In one of our patients, neither Doppler pressure measurements nor contrast arteriography detected a lesion of the popliteal artery that over the next 5 days led to the development of a large popliteal fossa pseudoaneurysm. This particular case emphasizes the importance of follow-up in patients who have suffered extremity trauma with potential vascular damage. Although it has been argued that the unpredictable compliance of most trauma victims mandates contrast arteriography at the time of their initial injuries (to assure that any occult vascular problems are identified "on the spot"),[17] others have suggested that, properly advised, such individuals *will* return for follow-up.[18] All of our 93 patients were urged to return to vascular clinic for examination and repeat Doppler pressure measurements: 88 (94%) returned at least once, albeit occasionally in involuntary fashion (either from jail[18] or after being rehospitalized for the consequences of recurrent interpersonal violence[19]).

Certain limitations inevitably attend the use of Doppler pressure measurement to detect occult arterial injury in traumatized extremities. Some injuries are so extensive that placement of a blood pressure cuff is not possible. Patients in shock may have unreliable peripheral arterial pressure determinations. Doppler pressure measurements will not detect isolated venous injuries or arterial disruption in nonaxial collateral beds (*e.g.,* the profunda femoris artery and its branches), and may be diminished during transient post-traumatic arterial spasm. Finally API must be considered only minimally reliable for more central injuries (*e.g.,* iliac and subclavian arteries), and in our subsequent analyses we have used this technique only for injuries distal to the axilla and the inguinal ligament.

Contrast arteriography in the trauma victim is expensive and time consuming. Further, certain patients suffer technical complications (dye toxicity, puncture site problems), or their diagnostic procedures result in falsely positive or negative studies. Such shortcomings are acceptable in direct proportion to the value of the arteriographic results obtained. In the damaged limb with minimal or no signs of arterial injury (especially where wound proximity is the primary concern), however, both our study and those previously reported[4-11,16] clearly demonstrate that screening arteriography usually is negative. In those uncommon circumstances when arteriography is positive, it often reveals clinically irrelevant arterial defects.

Therefore our demonstration that Doppler arterial pressure determination in extremity trauma appears to be as sensitive and specific for occult arterial injury as arteriography may have significant clinical implications. Significant savings in hospital charges and avoidance of the technical complications associated with arteriography might be realized. Substantial savings in time will surely expedite overall management of the trauma victim. And an efficient diagnostic protocol for extremity trauma can be proposed (Fig. 1). According to this algorithm, patients with extremity trauma and obvious signs of arterial trauma should undergo immediate exploration. Those patients whose extremities are devoid of evidence for arterial involvement could be "screened" by Doppler arterial pressure measurement. Arteriography would be reserved for those with abnormal results; patients with normal studies might simply be followed up by serial noninvasive examinations. Such a trial, in which patients with extremity trauma and an API > 0.90 are simply re-examined serially (including duplex scanning) in place of contrast arteriography, is underway at our institution.

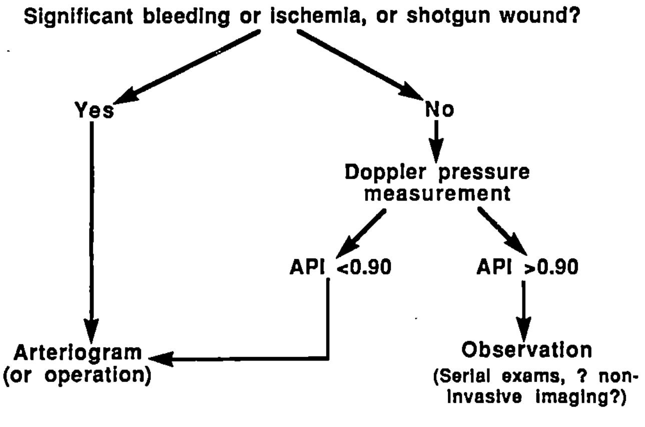
Significant bleeding or ischemia, or shotgun wound?
Yes
No
Doppler pressure measurement
API <0.90
API >0.90
Arteriogram (or operation)
Observation
(Serial exams, ? non-invasive imaging?)

FIG. 1. A proposed diagnostic algorithm for extremity trauma.

References

1. Perry MO, Thal ER, Shires GT. Management of arterial injuries. Ann Surg 1971; 173:403–408.
2. Snyder WH, Thal ER, Bridges RA, et al. The validity of normal arteriography in penetrating trauma. Arch Surg 1978; 113:424–428.
3. Dardik II, Ibrahim IM. Routine intraoperative angiography. Arch Surg 1975; 110:184–190.
4. Anderson RJ, Hobson RW, Padberg FT, et al. Penetrating extremity trauma: identification of patients at high risk requiring arteriography. J Vasc Surg 1990; 11:544–548.
5. Reid JSD, Weigelt JA, Thal ER, et al. Assessment of proximity of a wound to major vascular structures as an indication for arteriography. Arch Surg 1988; 123:942–946.
6. Rose SC, Moore EE. Trauma angiography: the use of clinical findings to improve patient selection and care preparation. J Trauma 1988; 28:240–245.

7. Frykberg ER, Crump JM, Vines FS, et al. A reassessment of the role of arteriography in assessing acute vascular injuries. J Trauma 1989; 29:1041–1052.
8. Dennis JW, Frykberg ER, Crump JW, et al. New perspectives on the management of penetrating trauma in proximity to major limb arteries. J Vasc Surg 1990; 11:85–93.
9. Weaver FA, Yellin AE, Bauer MAP, et al. Is arterial proximity a valid indication for arteriography in penetrating extremity trauma? A prospective analysis. Arch Surg 1990; 125:1256–1260.
10. Stain S, Yellin AE, Weaver FA, et al. Selective management of nonocclusive arterial injuries. Arch Surg 1989; 124:1136–1141.
11. Frykberg ER, Vines FS, Alexander RH. The natural history of clinically occult arterial injuries: a prospective evaluation. J Trauma 1989; 29:577–583.
12. Walker MC, Poindexter JM, Stovall I. Principles of management of shotgun wounds. Surg Gynecol Obstet 1990; 170:97.
13. Sirinek K, Levine BA, Gaskill HV, et al. Reassessment of the role of routine operative exploration in vascular trauma. J Trauma 1981; 21:339–344.
14. O'Gorman RB, Feliciano DV. Arteriography performed in the emergency center. Am J Surg 1986; 152:323–325.
15. Anderson RJ, Hobson RW, Padberg FT, et al. Reduced dependency on arteriography for penetrating extremity trauma (PET): influence of wound location and non-invasive vascular studies. J Trauma 1990; 30:1059–1065.
16. Bliss B, Mansfield AO, Shepherd R, et al. Vascular injuries (editorial). J Bone Jt Surg 1989; 71B:738.
17. Blaisdell FW, in discussion of Stain S, Yellin AE, Weaver FA, et al. Selective management of nonocclusive arterial injuries. Arch Surg 1989; 124:1136–1141.
18. Yellin A, in discussion of Stain S, Yellin AE, Weaver FA, et al. Selective management of nonocclusive arterial injuries. Arch Surg 1989; 124:1136–1141.
19. Sims DW, Bivins BA, Obeid FN, et al. Urban trauma: a chronic recurrent disease. J Trauma 1989; 29:940–947.

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- working with a team of well trained colleagues with whom they discuss clinical issues rather than reimbursement problems;
- quality cross coverage and built-in time off for a personal, family life;
- living in the Capital Region which offers multiple cultural, educational and recreational opportunities with easy access to Boston, Manhattan and Montreal.

For more information please contact:

Mary Brand or Lynn Harris
Physician Recruiters, Dept. S
Community Health Plan
1201 Troy-Schenectady Road
Latham, New York 12110
1-800-638-0668

CHAIRMAN DEPARTMENT OF SURGERY EASTERN VIRGINIA MEDICAL SCHOOL

Candidates are being sought for the position of Professor and Chairman of the Department of Surgery, Eastern Virginia Medical School.

The Eastern Virginia Medical School is a well-established community-based medical school with an enrollment of 370 medical students and 300 residents and fellows. The 250 full-time faculty and 800 volunteer faculty serve a population of more than 1,000,000 and are affiliated with all major hospitals in the area.

We seek a candidate with outstanding academic credentials who is Board certified and eligible for medical licensure in Virginia with demonstrated experience and skill to provide leadership in teaching medical students, residents and fellows, administration, research and service.

Submit curriculum vitae, summary of current research activities, goals, and research funding records, and the names of three or more references to:

David B. Propert, M.D., Chairman
Surgery Search Committee
Department of Internal Medicine
Eastern Virginia Medical School
825 Fairfax Avenue
Norfolk, Virginia 23507

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